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RON AND MANGANESE CONTENT OF CERTAIN SPECIES OF SEEDS[1]

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The occurrence of manganese and iron in the seeds of different species f plants has been noted by a number of investigators. However, there re but few data which show the amount of each of these elements conained in different species of seeds.

Headden[2] found that the amount of iron contained in the straw of vheat was two and one-half to six times the amount of manganese, while n the kernels the manganese is approximately equal to the iron and at he same time is higher, as a rule, than in the straw.

More recently Jones and Bullis[3] have shown that manganese is conained in considerable amounts in the aerial portions of certain leguminous plants, and from their results they conclude that alsike clover utilizes nanganese in larger amounts than any other legume commonly grown n the State of Oregon and that alfalfa makes the least use of it.

Since the writer[4] has obtained data which show that manganese is a necessary nutrient in the growth of plants, it therefore becomes a matter f interest to make a determination of the amount of iron and, manganese ontained in seeds of some species of plants and to correlate the esults obtained.

The results contained in this paper have been obtained on samples f seeds procured from several different parts of this country. Most of he samples of wheat were obtained through the courtesy of the Kansas nd Nebraska Experiment Stations and represent some of the more seful varieties grown in these and other States. A few of the samples f wheat and oats were obtained from the Departments of Plant Breeding and Farm Crops at Cornell University and had been grown on the xperimental plots at that institution. Most of the other seeds were btained from a seed company at Lexington, Ky., but the localities n which they were grown are not known to the writer.

The methods used in the estimation of the iron and manganese in he seeds were: For iron, the colorimetric thiocyanate method;[5] and or manganese, the colorimetric periodate method.[6]

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[1] Accepted for publication Oct. 16, 1922. Published with the approval of the Director of the Kentucky gricultural Experiment Station.

[2] HEADDEN, William P. OCCURRENCE OF MANGANESE IN WHEAT. *In* Jour. Agr. Research, v. 5, p. 349-5. 1915. Literature cited, p. 355.

[3] JONES, J. S., and BULLIS, D. E. MANGANESE IN COMMONLY GROWN LEGUMES. *In* Jour. Indus. and ngin. Chem., v. 13, p. 524-525. 1921.

[4] McHARGUE, J. S. THE ROLE OF MANGANESE IN PLANTS. *In* Jour. Amer. Chem. Soc., v. 44, p. 1592-1598, fig. 1922.

[5] SCOTT, Wilfred W., ed. STANDARD METHODS OF CHEMICAL ANALYSIS. p. 222. 1917.

[6] WILLARD, Hobert H., and GREATHOUSE, Lucien H. THE COLORIMETRIC DETERMINATION OF MANGANESE BY OXIDATION WITH PERIODATE. *In* Jour. Amer. Chem. Soc., v. 39, p. 2366-2377. 1917. Bibliography, p. 2376-2377.

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It is known that the presence of considerable phosphorus affects the accuracy of the colorimetric thiocyanate method for the estimation of iron. The following experiment was performed to determine whether the amount of phosphorus contained in seeds was enough to affect the estimation of iron by the thiocyanate method. Graduated portions of a standard solution made by dissolving 5 gm. of potassium phosphate (KH_2PO_4) in 100 cc. of water were measured out into a series of test tubes. The phosphorus contained in the different tubes varied from an amount much less than that found in seeds and gradually increased until the phosphorus content was several times this amount. To the tubes containing the different concentrations of phosphorus were added equal amounts of the necessary reagents including 2 cc. of a standard ferric iron solution, and the red color produced compared with the color developed in a tube containing an equal amount of all of the reagents used in the experiment except phosphorus.

No observable effect on the color of the solution could be detected until the phosphorus content was approximately .05 gm., an amount which is much in excess of that contained in an aliquot necessary for the estimation of iron in seeds. The color produced with thiocyanate in the presence of greater concentrations than .05 gm. of phosphorus was yellowish red instead of blood red as was produced with lower concentrations of phosphorus.

The results obtained represent the amounts of iron and manganese contained in the moisture-free seeds.

Table I.—*Percentages of iron and manganese found in the different species of seeds*

WHEAT

Variety.	Fe.	Mn.
	Per cent.	*Per cent.*
Big Flame	0.0031	0.0043
Dawson	.0036	.0049
Gold Coin	.0032	.0042
Gypsy	.0051	.0049
Deitz	.0043	.0038
Nebraska No. 60	.0032	.0061
Kanred	.0039	.0036
Red Wave	.0041	.0037
Ghirka	.0040	.0073
Mutant (Nebr. No. 28)	.0042	.0043
Average	.0039	.0047

SPRING OATS

Variety.	Fe.	Mn.
White	0.0046	0.0058
Do	.0049	.0048
Do	.0078	.0035
Black	.0062	.0038
Burt	.0034	.0048
Rust proof	.0052	.0060
Mixed	.0034	.0052
Victory	.0042	.0052
Average	.0050	.0049

TABLE I.—*Percentages of iron and manganese found in the different species of seeds*—Con.

GARDEN PEAS

	Fe.	Mn.
	Per cent.	*Per cent.*
Alaska	0.0070	0.0012
Telephone	.0080	.0010
Gradus	.0138	.0014
Average	.0096	.0012

GARDEN BEANS

	Fe.	Mn.
Tennessee Green Pod	0.0100	0.0017
Kentucky Wonder (brown seeds)	.0110	.0016
Kentucky Wonder (white seeds)	.0128	.0018
Pole Lima	.0080	.0018
Bunch Lima	.0100	.0019
Average	.0103	.0018

SOYBEANS

	Fe.	Mn.
3rooks	0.0057	0.0021
Aandarin	.0081	.0025
Aanchu	.0072	.0025
Kansas No. 1430	.0062	.0023
Haberlandt	.0088	.0033
Black Eyebrow	.0080	.0025
Chestnut	.0087	.0033
Sooty	.0061	.0023
Peking	.0074	.0041
to San	.0081	.0031
Average	.0074	.0028

CLOVERS

	Fe.	Mn.
Alsike	0.0517	0.0028
Red	.0021	.0038
White	.0020	.0022
White (sweet)	.0290	.0027
Yellow (sweet)	.0015	.0014
Crimson	.0060	.0029
Japan	.0300	.0155
Vetch	.0085	.0023
Alfalfa	.0100	.0012
Average	.0156	.0039

GRASSES

	Fe.	Mn.
Redtop	0.0160	0.0510
Timothy	.0061	.0072
Kentucky bluegrass	.0350	.0085
Orchard grass	.0070	.0176
Millet	.0052	.0017

TABLE I.—*Percentages of iron and manganese found in the different species of seeds*—Con.

GRASSES—continued

	Fe.	Mn.
	Per cent.	*Per cent.*
Corn (white H. K.)	.0017	.0007
Corn (yellow)	.0026	.0004
Cane (sorghum)	.0078	.0019
Average	.0107	.0111

MISCELLANEOUS

Sunflower	0.0034	0.0023
Hemp	.0210	.0165
Flax	.0085	.0038
Rape	.0059	.0046
Tobacco	.0240	.0070

In the foregoing results the following points of interest are worthy of mention.

In the samples of wheat the average percentage of manganese is slightly greater than that of iron, which shows that the capacity of this plant for storing manganese in the seed is as great as its capacity for storing iron. This fact suggests the possibility that manganese may perform a function of equal importance to that of iron in the plant's metabolism. In one-half of the number of samples the iron was slightly greater than the manganese in the same sample. The largest percentage of manganese was found in the Ghirka variety and the largest percentage of iron in the Gypsy.

In a previous article by the writer [7] it was pointed out that manganese occurs in greatest concentrations in the outer membranes of this seed and least in the starchy, glutinous material in the interior of the endosperm. It is therefore evident that in the processes of manufacturing the highest grades of patent flour most of the manganese is removed in the offal. Then, if it should be proved that manganese is also a necessary element in the diet, it is apparent that patent flour would contain less manganese than whole wheat or graham flour.

The average results for iron and manganese in oats show that these two elements are more nearly equally distributed than in wheat.

In the preparation of oatmeal for human consumption the manganese would not be eliminated in the offal to the extent it is in the manufacture of flour from wheat. Therefore oatmeal affords one of the richest sources of manganese in food, and it is quite probable that it does add some nutritive value to this material.

The average results for iron and manganese in garden peas and beans are approximately the same in each of the different kinds of seeds. It is also of interest to note that the iron content is more than six times the amount of manganese in these seeds. The iron content in peas and beans is almost three times that found in wheat and twice the amount

[7] MCHARGUE, J. S. THE OCCURRENCE AND SIGNIFICANCE OF MANGANESE IN THE SEED COAT OF VARIOUS SEEDS. *In* Jour. Amer. Chem. Soc., v. 36, p. 2532–2536. 1914.

contained in oats. The manganese content of wheat and oats is approximately three times that contained in peas and beans.

In 10 different varieties of soybeans the average percentage of iron was nearly three times as large as the average percentage of manganese. Since soybeans have some use as a food and feed material it is of interest to note that they supply a relatively large amount of iron in the diet. Soybeans contain less iron than garden peas and beans, but more manganese.

The average results for nine varieties of clover show an iron content of nearly four times that of manganese. The average percentage of iron is about twice the iron content in soybeans, while the manganese content is about the same. Alsike clover contains nearly 20 times as much iron as manganese. While white sweet clover contains a little more than 10 times as much iron as manganese, yellow sweet clover contains nearly equal amounts of each of these elements. Both the white and yellow sweet clover seeds had been decorticated. The iron content of Japan clover is about twice that of manganese. The clovers contain considerably more iron than any of the other species of seeds examined.

In the grasses, redtop contained more than three times as much manganese as iron, while bluegrass contained more than four times as much iron as manganese. Orchard grass contained more than twice as much manganese as iron.

White and yellow field corn contained the smallest amounts of iron and manganese of any seed examined. Since most of the iron and manganese contained in corn is found in the germ and bran and since the food products of corn as they are prepared to-day exclude these parts of the grain, it is evident that such food products would be practically a negligible source of manganese.

Hemp seed contained relatively large and nearly equal amounts of iron and manganese. The largest amounts of iron were found in Alsike clover, bluegrass, Japan clover, hemp, and redtop, respectively, while the largest amounts of manganese were found in redtop, Japan clover, orchard grass, hemp, and bluegrass, respectively. Japan clover seed retains the calyx and pod, which may account for its high manganese content.

It is to be noted that the manganese content is much less than the iron content in legumes, whereas in cereals the iron and manganese contents are more nearly equal.

Seeds containing the most iron did not contain the largest amounts of manganese. In the grasses there appears to be considerable fluctuation in the iron and manganese content in one variety as compared with another. Thus manganese is in excess in redtop while iron predominates in bluegrass.

CONCLUSIONS

The average manganese content of the seeds of wheat and oats produced under natural conditions in the soil is equal to the average amount of iron found.

The amount of manganese in the seeds of legumes is much less than the iron content.

The amount of iron and manganese found in grasses showed considerable fluctuations with varieties, but the average results are nearly equal.

STUDIES UPON THE LIFE CYCLES OF THE BACTERIA—PART II: LIFE HISTORY OF AZOTOBACTER [1]

By F. LÖHNIS, *Soil Bacteriologist*, and N. R. SMITH, *Assistant Bacteriologist, Bureau of Plant Industry, United States Department of Agriculture* [2]

INTRODUCTION

In a preliminary communication, published in this Journal in 1916 (*28*),[3] it was pointed out (1) that the life history of Azotobacter is much more complicated than was generally assumed and (2) that the same holds true with regard to all other bacteria. The correctness of this general statement was further demonstrated by the senior author in a critical review of the bacteriological literature, published as Part I of these Studies (*25*). It was shown therein that all findings briefly recorded in our preliminary communication are in complete agreement with numerous analogous observations made between 1838 and 1918. That these older findings had failed to lead to a more accurate knowledge of bacterial pleobiosis was caused by their being widely scattered in a gigantic literature and by their being not in accordance with time-honored, though incorrect, theories concerning bacterial monomorphism and constancy.

Since Part I was written, several contributions have been published which further support our standpoint. Bergstrand (*4, 5*) studied the wide pleomorphism of a yellow diphtheroid and advocated that all bacteria, on account of their budding, branching, etc. should be classed among the Fungi Imperfecti. Brown and Orcutt (*7*) recorded bacillary, fusiform, filamentous branching, diphtheroid, and streptococcoid growth of *Bacterium pyogenes*. Thompson (*42*) noticed that *B. proteus*, when grown in symbiosis with *B. tuberculosis*, with other mycobacteria or actinomycetes, changed to a diphtheroid, anaerobic organism in 62 per cent of all cases. A fuso-spirillary organism was seen by Mellon (*31*) to grow (1) as a yellow actinomyces-like organism, (2) as a diphtheroid, making small semitransparent colonies, (3) as a white, aerobic, gelatine liquefying coccus, (4) as a typical anaerobic Fusiformis, and (5) as an anaerobic spirillum-like organism; filterable gonidia, "giant cocci" (evidently gonidangia) and phases of the symplastic stage were also recorded. By the same author (*32, 33*) the "giant cocci" of *Corynebacterium Hodgkinii* were isolated and stabilized, and their "schizogony," dividing, budding and germination were carefully studied. Wade and Manalang (*44*) made an interesting report upon the various growth forms of *Bacterium influenzae*, wherein they state:

> The familiar bacillus is but a simple form of an organism capable of complex development.

[1] Accepted for publication Sept. 26, 1921.

[2] We are indebted to Mr. F. L. Goll, of the Bureau of Plant Industry, for making the photomicrographs reproduced on the plates attached to this paper.

[3] Reference is made by number (italic) to "Literature cited," p. 430–432.

They are inclined to place it therefore among the Actinomycetes, but a comparison of their findings with those discussed in Part I of our Studies (*25*) leaves no doubt that *Bacterium influenzae*, too, follows exactly the same general lines which are recognizable in the life history of all bacteria. The general occurrence of filterable gonidia is further indicated by observations made by Heymans (*13*) with regard to anthrax and tuberculosis. It was found that the bacteria pass the filter in an ultramicroscopic form. Hort (*14*) published additional details concerning the various modes of bacterial reproduction; Howe and Hatch (*15*) confirmed Noguchi's finding that *B. bifidus* can grow as an aerobic, sporulating bacillus; and Orla-Jensen (*38*) pointed out that certain aerobic plectridia can be easily transformed so that they are no more distinguishable from the typical Proteus bacteria. Studies upon the conjunction of bacterial cells were made by Potthoff (*39*) with Chromatium and sulfur spirilla, and by Enderlein (*8*) with *Vibrio cholerae*. The results obtained are in good agreement with earlier findings (*25, p. 195, 202*). The same holds true in regard to more recent observations made by Lutz (*30a*) upon the different types of globoid bodies produced by *B. anthracis*. Almquist (*2a*) and Mellon (*33a*) have again emphasized that a thorough knowledge of the various developmental phases in the life histories of pathogenic bacteria is essential for gaining a correct view of epidemiological problems.

Our observation upon the life cycle of Azotobacter, as recorded in our preliminary paper (*28*), were partially confirmed and partially contested by D. H. Jones (*16*). Confirmed were the data on pleomorphism and symplastic stage, contested those on conjunction and on the ability of Azotobacter to assume endospore formation. The discussion of these differences of opinion will be taken up at the end of this report after our experimental results, obtained since 1916, have been presented.

METHODS OF INVESTIGATION

To obtain complete and accurate information upon the life cycles of the bacteria is no more difficult but much more time-consuming than to make an ordinary bacteriological diagnosis. Single-cell cultures and continuous microscopic observation of the living organisms are by no means so absolutely indispensable as is sometimes asserted (*25, p. 39, 205*). The generally used methods of isolating, cultivating, and studying the bacteria are, as a rule, quite sufficient to collect complete information, provided they are applied judiciously and the investigator himself is not too preoccupied by the wide-spread prejudices concerning "normal" and "abnormal" growth, "involution forms," and "contamination".

A sufficient number of parallel tests, the frequently repeated microscopic control of the cultures held for a sufficient length of time (not less than a month, preferably longer), and the regular repetition of all experiments are three points of major importance. Accordingly, apparent changes of growth can be correctly accepted as such, only if they have been ascertained repeatedly in parallel tests and if they have been closely followed under the microscope. Furthermore, an apparently new type of growth must always be tested in regard to its inclination to return to its origin, or to pass over into another well-known developmental stage of that particular organism. Sometimes the patience and persistence of the investigator is taxed very much by such experiments; it may take years before positive results are secured. A few examples in this respect were

mentioned in Part I (*25, p. 29, 34*) and others will be discussed on the following pages. On the other hand, the instability of a new form may prove vexatious. Even if a new type of growth is represented by a sufficient number of cells in a culture, and its separation by plating therefore appears to be promising, nevertheless a complete failure may result, due either to an immediate return to the original type of growth or to the disinclination of the new form to grow in pure culture on the substrate used. Occasionally the stabilization of the new form proceeds very slowly and gradually; its colonies may appear very late on the plates, or they may at first simulate those of the mother form so closely that only microscopically can a differentiation be made. However, these particular difficulties are rather an exception to the rule; usually the new type of growth will be isolated if it is represented in the original culture by a sufficient number of cells. To reduce the chances for contamination as far as possible we prefer for plating flat bottles with a neck not much wider than a test tube. The dilutions are made directly in the bottles wherein the substrate was previously sterilized.

Besides beef agar, beef gelatine, beef broth, milk, and potato the substrates most frequently used in our Azotobacter experiments were mannite-nitrate solution and mannite-nitrate agar. The composition of these media was given in our preliminary communication (*28, p. 686*). Our recipe was changed by E. R. Allen (*2*) with very unsatisfactory results; it is, however, only his substrate, not, as he asserts, the "medium of Löhnis and Smith" which proved to be unsuitable for Azotobacter growth. We studied for example, the alternation between the varied cell life and the symplastic stage of this organism in the original cultures, made in mannite-nitrate solution, for more than a year and then kept these cultures four years longer (most of the time sealed); transferred to new solution at the end of the whole period, 60 per cent of the cultures gave new vigorous growth. The hydrogen-ion concentration of these substrates was usually kept approximately at P_H 6.8; occasionally it was increased to 6.0 or lowered to 7.5–8.0. Potato agar (15 gm. agar and 10 gm. peptone added to 1,000 cc. filtered potato water, prepared by boiling 200 gm. potato in 1,200 cc. water, to which an excess of $CaCO_3$ was added) was also found to be very useful, as was the case, although less frequently, with soda agar (beef agar with ¼ per cent Na_2CO_3), phosphate agar (beef agar with ⅛ per cent $Ca(H_2PO_4)_2$), salt agar (beef agar with 2 to 8 per cent NaCl), and water to which 5 cc. beef broth per 1,000 cc. were added. Most instructive results, however, were secured from cultures kept in sterilized soil (about 5 gm. in test tubes heated in the autoclave for 1½ hour at 20 pounds pressure) moistened with 0.5 per cent mannite solution. Within a few weeks all developmental stages were passed in this substrate with greatest regularity.

Some experiments upon the effect of symbiosis of Azotobacter and Radiobacter were also included. It was pointed out in an earlier paper (*26*) that especially the regeneration of large spore-free Azotobacter cells from the sporulating growth is favored by the presence of Radiobacter.

The following cultures of Azotobacter were used in our experiments:

A. chroococcum Beij. (Laboratory Numbers 1, 2, 10, 11, 12, 14, 17 to 25).

A. Beijerinckii J. G. Lipman (Laboratory Numbers 3 to 6, 13, and 15).

A. agile Beij., syn. *A. Vinelandii* J. G. Lipman (Laboratory Numbers 7 to 7c, 16 to 16c).

A. vitreum Löhnis (Laboratory Number 9).

A. sp. (Laboratory Numbers 26 and 27).

Cultures No. 1 to 11 are identical with those studied in 1914 (*26*) with the exception of No. 7b and 7c, two strains of *Azotobacter agile* recently received from Kral's Museum in Vienna (*A. Vinelandii* Lipman-Ambrož and *A. agilis* Dahlem). No. 12 and 13 are old stock cultures of the Laboratory of Soil Bacteriology. No. 14 to 16 and 16c were received from Dr. J. G. Lipman, New Brunswick, N. J.; No. 16b, 17, and 20 are subcultures of No. 112, 141, and 522 of the New York Museum of Natural History. No. 18 and 19 were received from Prof. D. H. Jones, Guelph, Ontario, in 1912 (his strains 1 and 3). No. 21 to 25 are new isolations obtained in the spring of 1916 from soil samples received from Dr. C. B. Lipman, Berkeley, Calif. No. 26 and 27 were sent to us by Dr. M. Mulvania, Knoxville, Tenn.

Fifteen of these strains (No. 7, 9, 10 to 14, 16, 16c, 18, 21 to 25) grew as typical large nonsporulating cells, when the investigations were started; 4 cultures showed only large sporulating cells (No. 2, 3, 4, and 7b), 2 small sporulating rods (No. 5 and 6), and 3 irregular fungoid cells (No. 15, 17, and 19); 4 were made up of small nonsporulating rods (No. 7c, 16b, 26, and 27), 1 of coccoids (No. 20), and 1 of dwarfed cells (No. 1). Although it was known in only some of the cases where atypical growth occurred that this was not due to contamination, we decided to include all atypical strains in our studies and to base our ultimate decision upon the outcome of these experiments.

Each of the 30 Azotobacter strains was tested in about 100 to 200 and sometimes more transfers. Each transfer was subjected to 4 to 6, but occasionally to many more microscopic tests. The results presented in this paper are therefore based on over 20,000 observations. Numerous additional tests were made with cultures of several other "species," which proved to be identical with the newly evolved growth types of Azotobacter—namely *Bacillus petasites* A.M. et Gottheil, *Bacillus malabarensis* Löhnis et Pillai, *Bacillus danicus* Löhnis et Westermann, *Bacillus pumilus* A.M. et Gottheil, *Bacillus Freudenreichii* (Miquel) Mig., *Bacillus fusiformis* A.M. et Gottheil, and *Bacterium lactis viscosum* (Adametz) Lehm. et Neum. Some of these cultures were kept in the senior author's collection; others were obtained from New York or from Vienna.

LIFE CYCLE OF AZOTOBACTER

In Figure 1 of our preliminary paper (*28*) we gave a schematic sketch of the various cell types and modes of reproduction of Azotobacter. Four types or subcycles of growth were considered to be most characteristic. The more thorough study of the problem, however, led to the discovery that from every Azotobacter culture not less than seven different growth types can be developed and stabilized; all of them are interchangeable. The cells characterizing these seven types of growth are the following (the letters in parentheses referring to the designations used in our preliminary paper):

Large non-sporulating cells (types A, B, Lα, Kα, and J).

Coccoid forms (type I).

Dwarfed cell type (types Eα, Eβ, and Kϵ).

Fungoid cell type (types G and Kγ).

Small non-sporulating rods (type Fα).

Small sporulating rods (type Fβ).

Large sporulating cells (types L, M, and Kλ).

Inspection of figure 1 of the preliminary paper (*28*) will show that the increase in the number of growth types from four to seven is due to the fact that types I and G (with Kγ) could also be stablized, and that the three types E, Fα, and Fβ could be grown separately.

In accordance with the arrangement made in Part I of these Studies (*25*) the different cell types, the various modes of reproduction, the symplastic stage, and the conjunction of vegetative and reproductive cells will be discussed consecutively. The cultural characteristics of the different developmental stages, however, will be given separately in a special chapter. The morphological features of vegetative and reproductive cells were found to be fundamentally the same with all our Azotobacter strains, while the cultural peculiarities allow a clear separation of the four species (or varieties) *Azotobacter chroococcum*, *A. Beijerinckii*, *A. agile*, and *A. vitreum*. Accordingly, the morphological facts observed with the different strains will be considered jointly, while the cultural characteristics of the four groups will be discussed separately.

I.—DIFFERENT CELL FORMS OF AZOTOBACTER

The wide pleomorphism of Azotobacter was discussed to some extent in earlier publications (*3*, *9*, *26*, *30*, and *40*). But how really bewildering the multitude of cell types is which this organism is able to produce in the course of its life cycle may be gathered from the photographs reproduced on the plates attached to this paper. Even they, of course, do not exhibit all forms which were observed in the course of our studies. But combined with the data presented in our preliminary report (*28*) and in Part I (*25*) they will suffice to indicate that an approximately complete picture of the form cycle of a bacterium is very different from the customary meager description of a so-called bacterial species.

The relatively large globules, ovals, or rodlike cells, measuring about 2 to 4 by 3 to 5 to 7μ, motile either (ovals) by polar or (rods) by peritrichous flagella, mostly Gram-negative, are in most cases very unstable. Figures 1, 2, 4, and 6 to 8 of Plate 1 demonstrate how even in young cultures (2 to 7 days old) the typical large cells are inclined to change to small globular forms (coccoids) or to more slender pointed rods, characteristic of the sporulating type of growth. In older cultures large and small rods, irregular fungoid, as well as very small dwarfed cells are always present, as is shown in figures 3, 5, 9, 11, and 12 from growths 3 weeks to 11 months old. All these forms are viable, and therefore not to be classed as involution forms. Figures 1 to 5, 9, and 11 make it fairly clear that the decrease in cell size is largely due to a loss of voluminous slimy cell elements and to the liberation and multiplication of nuclear material, which according to the quality of accessory substances presents itself as either well or weakly stainable, or as entirely unstainable when treated with aqueous dyes. Potato agar favored especially the appearance of globular cells; slightly acid mannite-nitrate agar (P_H 6.0) strengthened the tendency to produce small rods and coccoids. Only two of our cultures (No. 13 and 18) grew permanently on all substrates in the large typical form, but also in these cases the microscope always revealed the presence of the other cell types in small number.

The varying appearance and behavior of the coccoid cells of medium and of small size is illustrated on Plate 2. Figures 13 to 15 demonstrate their budding out of the larger cells; figure 16 shows multiplication by budding and by fission; figures 17, 18, and 21 represent the fairly uniform

growth of the coccoids, which were always found immotile and Gram-positive. Figures 19 and 20 illustrate the upgrowth to larger globular cells, and figures 22 to 24 the streching to small and large non-sporulating and sporulating rods. The coccoids shown in figures 17 and 18 look very much like typical micrococci, and they may, in fact, assume a great stability of growth. But sooner or later they, too, will either return to the large cell form or elongate to regular or irregular rod forms. One of our strains (coccoids of *Azotobacter agile*, No. 7) remained fairly stable as a pink "micrococcus," for not less than five years in 71 transfers, and then it returned to the large original growth type only if kept either in milk or in mannite-nitrate solution for one to two months. Milk and potato agar were usually most favorable for a return to the large cell type, while beef agar, beef broth, and mannite-nitrate solution induced the develpment of rodlike and fungoid forms.

The dwarfed cell type, first discovered in an old culture of *Azotobacter chroococcum* (*26*), can be evolved from Azotobacter cultures as easily as or even more easily than the occcoid growth. It is either immotile and Gram-negative or (the smallest units) motile and Gram-positive. The liberation of these smallest specks of mostly nuclear material from the larger cells (by budding or by so-called granular decomposition) can be seen in every culture (fig. 3 and 5 of Pl. 1). But separate development is usually slow and inconspicuous, and the isolation of this type of growth, therefore, not always easy. Pure cultures once established, however, are much inclined to reproduce larger cells, especially rods and fungoid growth. Figures 25 to 36 on Plate 3 show the typical growth and the alterations most frequently observed. A close study of the minute forms characteristic of this stage (fig. 25 and 26) reveals the tendency to present a more or less irregular, often wedge-shaped appearance. This tendency becomes more noticeable when the upgrowth takes place either by a uniting of the small granular bodies in slimy threads (fig. 28 and 29) or by their stretching to small rodlike cells (fig. 30 and 31). On the other hand, very regular, weakly staining small ovals may appear, or the small irregular cells may assume globular shape (fig. 27), simulating in this case small micrococci. The latter process is always ascertainable in cultures several weeks old, whose miscroscopic appearance is very similar to that shown in figure 18 on Plate 2. Large rod-shaped, threadlike, or large globular cells are comparatively rare in cultures of this type; if they appear they are probably always produced by the symplasm (fig. 32 to 36). The simultaneous reproduction of small cells, of long slimy threads, which are occasionally branched, and of large ovals and globules deserve special attention (fig. 33 and 36; both pictures made from the same slide). The gradual upgrowth of rods from small to large size (shown in fig. 34) is equally of great interest. The pale, small ovals, mentioned above, seem to be able to assume directly the qualities of an endospore, able to reproduce a fairly large sporulating rod. This transformation was repeatedly observed with our cultures No. 1 and 2.

The fungoid cell type is represented by figures 37 to 48 on Plate 4. Figures 37 to 39 illustrate that this irregular cell type may in fact assume distinctly fungoid appearance; figure 11 on Pl. 1 deserves also renewed inspection. Figures 37 and 38 picture the cells most frequent and most typical; they are made up of unstainable slime and darkly staining granules of nuclear material. Temporarily this slime is soluble in boiling water, and therefore the fungoid type of growth was not recognized as such in our preliminary report (*28*). But from the unstainable

granulated slime threads of the dwarfed cell type shown in figures 28 and 29 on Plate 3, the development proceeds over the steps illustrated by figures 37 to 39 on Plate 4 further to a very stable type of irregular, fungoid growth, which ultimately acquires all the qualities characteristic of a Mycobacterium. Wedge-shaped cells, budding and branching, club formation, etc. are all noticeable, and the same holds true concerning the cultural features of this "genus". The clubs again are no "involution forms," but steps in the development of rods with terminal spores (fig. 45). Dwarfed cells, coccoid forms, rods of various size, as well as large non-sporulating globules and ovals were all evolved from this type of growth, as illustrated by figures 40 to 48 (Pl. 4). Cultivation on potato was found useful for stimulating the tendency to produce clubs and rudimentary endospores. Potato agar favored again the development of coccoid forms. In water, broth, and milk branching was very pronounced, but continued cultivation in milk established a growth of regular spore-free, slime-producing rods. Alkaline mannite-nitrate solution and agar (P_H 7.5) proved helpful for reestablishing the typical Azotobacter growth. It is especially noteworthy that this was secured on these substrates with one of our cultures of *Azotobacter Beijerinckii* (No. 15) which had grown for six years in 145 transfers on practically every substrate as a highly pleomorphous mycobacterium. It had been plated repeatedly, strains representing the dwarfed growth, coccoid growth, sporulating and non-sporulating rods had been branched off, until ultimately after a passage on potato agar (fig. 44) the restoration of the original Azotobacter type was effected (fig. 47).

The typical form of the small spore-free rods of *Azotobacter chroococcum* and *A. Beijerinckii* is shown in figures 49 and 50 on Plate 5, while those of *A. agile* and *A. vitreum* are usually of more slender shape, similar to that type of growth of *A. chroococcum* visible in figure 51; fairly typical rods of *A. agile* are also to be found in the left upper corner of figure 60 on Plate 5. All these rods are Gram-negative, those of *A. chroococcum* and *A. Beijerinckii* immotile, those of *A. agile* and *A. vitreum* motile by usually three polar flagella. It should be noted that to start the cultures reproduced in figures 49 and 50 the same inoculation material was used; the difference in the substrates was the only cause of the very marked difference in appearance. A comparison of figure 49 with figure 9 and figure 24 will demonstrate the gradual changes from the large to the small non-sporulating cells, while the small coccobacilli in figure 50 should be compared on the one side with the dwarfed growth shown in figures 25 and 26 on Plate 3 and on the other hand with the short rod forms which always accompany the mycobacterium type, as demonstrated in figures 40 to 45 on Plate 4. The larger rod form producing a short branch, which is visible in the lower right corner of figure 50, is also of interest in this connection. A direct transformation from the original cultures of *A. chroococcum* and *A. Beijerinckii* to small non-sporulating rods was recorded only three times, while they were more frequently derived from the fungoid growth (nine cases observed). *A. agile* and *A. vitreum* behaved differently; several changes from the large cells to small rods, but no direct transformation from the fungoid growth could be ascertained. Figures 51 to 53 on Plate 5 demonstrate the trans formation from the short non-sporulating rods to coccoid and fun_ goid growths, the latter identical with that of figures 43 and 45 on Plate 4. That sporulating bacilli which have lost their ability to

produce regular endospores may display a very similar morphology may be derived from a comparative examination of figure 54 with figures 51 and 53. *Bacillus pumilus*, shown in figure 54, is identical with the sporulating rod form of *A. chroococcum*, as will be discussed presently. The return from the small rods to large globular cells is illustrated by figures 55 to 57; figure 58 pictures the transformation into the typical fungoid growth, similar to that of figures 37 and 38 on Plate 4, and in figures 59 and 60 the simultaneous upgrowth of small and large rods from the symplasm is demonstrated, figure 59 making a counterpart to figures 34 and 35 on Plate 3.

The small sporulating rods are of special interest because, when the endosporulation first becomes noticeable in an Azotobacter culture, nearly always these small rods, not the large sporulating cells, are the first ones to appear. Referring to what was said in Part I (*25, p. 137, 139*) about the transformation of terminal regenerative bodies into polar exospores and endospores, it is evident that this observation is in full agreement with all earlier findings. On the other hand, it must be pointed out that these rather fragile, Gram-negative, slender rods with large polar spores are by no means a frequent occurrence in our laboratories. To discard them lightly as "contaminations" would be entirely unjustified. Identical strains were isolated from various Azotobacter cultures in Leipzig, Washington, D. C., Urbana, Ill.; and a culture recently received from Vienna gave again the same small sporulating rod. But never in our experience, and especially not in any one of approximately 5,000 transfers made in the course of these experiments, were such sporulating small rods found as contaminations. On account of their weak growth we even lost two of them by replating; but in view of this experience it is especially interesting that in old Azotobacter cultures kept undisturbed for years on mannite-nitrate agar or in mannite-nitrate solution just these small sporulating rods were the only survivors. Figure 62 on Plate 6 shows such a strain, which should be compared with the typical picture reproduced as figure 61. Figures 63, 71, and 72, on the other hand, illustrate the tendency to pass over into large sporulating rods. That transformations from small to large sporulating cells were seen occasionally in our preliminary experiments was mentioned before (*28, p. 681*). But as the first appearance of these small sporulating rods caused considerable surprise in 1912 (*26*), so it was again the case when one of these cultures (No. 6), after having displayed a very constant behavior, ultimately in the spring of 1921, when cultivated on alkaline mannite-nitrate agar, reverted to the large-cell type and assumed gradually all the characters of the original strain (No. 3), from which it had been branched off nine years before. A tendency to change to the fungoid growth is noticeable with some of the cells in figures 64. Figures 65 to 70 illustrate certain reproductive phases to be discussed on the following pages.

The large sporulating cells are shown in figures 73, 74, 76, and 77 on Plate 7 in what may be accepted as their typical appearance. They are motile by peritrichous flagella and are Gram-positive. The tendency of the threads, very common in the cultures of this type, to make short, oval, Gram-negative cells is very pronounced. Figure 78 illustrates the analogous behavior of a thread in a spore-free Azotobacter culture. In figure 75 many branched rods and threads are visible, representing the fungoid type of the large rod form, which, however, could not be stabilized, although with *Bacillus anthracis*, *B. subtilis*, and *B. mycoides*

positive results had been obtained (*25, p. 62–64*). Figure 80 deserves special attention because it illustrates a mode of fission which has been observed by very few authors and which thus far has never been photographed. Large round bodies appear, which later split on their diameter, beginning in the center, into two large, comma-shaped bacilli so characteristic of *B. Megaterium* and related "species." As was said in Part I (*25, p. 125, 126*), Schroen has described this occurrence more than 30 years ago, and at the same time E. Klein and Dowdeswell made analogous observations with *Vibrio cholerae*. The small non-sporulating rod forms of Azotobacter seem to act occasionally in a similar manner. In figure 55 on Plate 5 an intact globule is visible and also several pairs of slightly curved, plump rods, of which especially those located near the upper edge of the photograph are very suggestive. Figure 84 on Plate 7 illustrates the return to the normal large spore-free cells (after passage of the symplastic stage). In potato cultures this reversion occurred most frequently. The cells shown in figure 79 and 81 to 83 are related to reproductive processes; they represent various types of gonidangia and sporangia.

2.—DIFFERENT MODES OF REPRODUCTION

All types of bacterial reproductive organs have been found with Azotobacter—namely, gonidia and gonidangia, regenerative bodies (zygospores, etc.), arthrospores, microcysts, endospores, and exospores. It was pointed out in Part I (*25, p. 119–143*) that all these organs of reproduction are fundamentally not so different as might be assumed. As the pleomorphism of the vegetative bacterial cells finds its explanation in the varying participation of nuclear material and of other cell elements in cell construction, so also in the various modes of reproduction nuclear substances always play the dominant rôle, supported to a smaller or larger extent by reserve material, cell membrane, etc. When the nuclear material is accompanied by very little other cell elements, gonidia are produced. More reserve material and a more or less durable membrane characterize the regenerative bodies and arthrospores. When the whole cell assumes a globular or oval shape and thickens its membrane a microcyst results, while the contraction of most of the cell content and the formation of an exceptionally tough membrane leads to the formation of an endospore, or of an exospore if the growing spore buds out of the mother cell. Gonidia and regenerative bodies may multiply as such, producing the dwarfed and the coccoid growths discussed above; arthrospores and microcysts, endospores and exospores are true resting forms, although a secondary transformation to regenerative bodies or to gonidia was also observed repeatedly with these types of reproductive organs. While bacteria of the usual small dimensions as a rule form only one to two to four gonidia in each cell, the larger cells, for which the term gonidangia was introduced in Part I (*25, p. 121*) may contain a much larger number or in their place two to three or more endospores; in the latter case the gonidangium becomes a true "sporangium."

Figures 85 to 87 on Plate 8 demonstrate formation and liberation of the gonidia by normal large globular Azotobacter cells, while figure 77 Plate 7 illustrates the analogous process with the large threads of the large sporulating type. A comparison of figures 86 and 87 shows how either (in the latter case) the nuclear material practically alone may persist and give rise to the dwarfed growth discussed above, or (in the former case) how other cell elements may participate in the

regeneration of larger, in this case of coccoid cells. (See also figure 5 on Plate A of our preliminary paper, (*28*) where both processes became visible simultaneously, and figures 3 and 4 on Plate 1 of the present paper.) In figure 77 on Plate 7 the two granulated threads in the lower left part of the field deserve special attention, because the regular arrangement of the unstained gonidia contained therein is very characteristic of Azotobacter, as is the stainability of the same bodies as soon as they have left their mother cell. (See especially the dark bodies budding from several threads in figure 77 and analogous occurrences photographed in figure 60 on Plate 5.) Other types of budding, as well as the transformation of regenerative bodies into larger cells, are pictured in figures 13 to 16, 22 to 24 on Plate 2. Figure 65 on Plate 6 demonstrates the formation of gonidia in the small sporulating cells, and also their development to regenerative bodies and to endospores.

Typical gonidangia of Azotobacter may be seen in figures 5 and 11 of Plate 1 and in figures 88 to 92 on Plate 8, those of the sporulating cell type in figures 79, 81, and 82 on Plate 7; in the latter case the swollen ovals are replacing the sporulating cells, while in the two former instances the globular, branched, or spindle-shaped cells are acting as true sporangia. If small cells swell up to gonidangia they look much like regular Azotobacter cells (fig. 20 on Pl. 2 and 57, on Pl. 5), and as the latter are always able to produce numerous gonidia, it seems as if the true physiological reason for the occurrence of this developmental stage lies in the ability of these cells to act as gonidangia. The peculiarity of all bacterial gonidangia to assume either globular, oval, club-shaped, or thread-like forms is also characteristic of the large spore-free cells of Azotobacter. That they frequently multiply as such without acting as reproductive organs places them parallel to the dwarfed growth (of the gonidia) and to the coccoid growth (of the regenerative bodies). Instead of gonidia or endospores new cells of normal size may be directly formed within these large receptacles. Figures 6 on Plate A and 21 on Plate D of our preliminary paper (*28*) illustrated such cases; similar preparates are shown in figures 90 to 92 and 94 on Plate 8 of the present paper.

The fungoid type of growth was shown to be the result of a consolidation of slime threads containing numerous gonidial bodies. Accordingly it is easy to understand why in this case the formation of arthrospores is very common. The gonidia simply increase somewhat in size at the cost of the cell and surround themselves separately with cell walls. Occasionally, but much more rarely, normal rods or threads act in an analogous manner. Figures 51, 54, and 55 on Plate 5, and figure 66 on Plate 6 illustrate the arthrospore formation of the small non-sporulating and sporulating growth types. An upgrowth of other globular cells from the encapsulated symplasm also visible in figure 66 will be discussed later. The fragmentation of the large threads shown in figure 78 on Plate 7 could also be accepted as another instance of arthrospore formation, but as the globular cells produced in this case are able to multiply as such, this occurrence is to be considered more similar to that pictured in figure 21 on Plate D of our preliminary paper (*28*) and mentioned above.

Terminal swellings—that is, regenerative bodies in a polar position, but still inclosed in the mother cell—are the first step toward the formation of endospores, as was fully discussed in Part I (*25*) and frequently confirmed in the course of these investigations. Figures 45 and 46 on Plate 4,

52 to 54 on Plate 5, as well as figure 83 on Plate 7, represent the characteristic picture of cultures inclined to begin or to resume endosporulation. Heating of such strains first to 70° C., later to 75°–90° C., provided that the tendency to produce such terminal bodies could be stabilized, leads eventually to the production of typical polar endospores or exospores. Negative results are frequent in such experiments, but they do not disprove the less numerous positive ones. As was pointed out before, these newly evolved small sporulating strains often grow very slowly and weakly, a fact which also deserves full attention. When completely developed, the endospores of the small-cell type withstood 95° C. for 4 to 5 minutes, 98° C. for 1 minute, and those of the large rods 98° C. for 2 to 4 minutes; the germination was mostly polar in both cases. Budding exospores were most clearly visible with the large sporulating rods, as was illustrated by figure 20 on Plate D of our preliminary paper (*28*). The spores do not always germinate directly; sometimes they first swell up to fairly large weakly staining ovals which are indistinguishable from microcysts. On the other hand it is no rare occurrence, especially when the small sporulating rods are cultivated in broth or milk, that the normally produced spores do not germinate but reproduce, either by budding or by dividing, 2 to 4 gonidia or regenerative bodies, which again may multiply as such (fig. 70 on Pl. 6). As was pointed out in Part I (*25, p. 141*), similar observaions were made before; they demonstrate anew that the formation of gonidia is in fact the fundamental mode of bacterial reproduction.

The transformation of vegetative cells to microcysts is most conspicuous with the large-cell types. The large sporulating rods produce weakly staining ovals (sometimes discarded as "shadows") which germinate readily to darkly staining, short, broad, mostly pointed rods (fig. 74 on Pl. 7). With the large spore-free cell type the microcysts are equally common; here they are the well-known thick-walled cells of globular, oval, or pointed shape, frequently united to two or four, whose germination was carefully studied by Prazmowski (*40*) and others. That the germ may be of coccoid or of threadlike shape is illustrated by figures 93 and 94 on Plate 8. With the small-cell types microcysts are less conspicuous. The coccoids are either thin-walled or thick-walled; in the latter case they may act as microcysts. With the dwarfed growth small pale ovals are not infrequent, perhaps a counterpart to those of the large sporulating cells. The large microcysts of the spore-free as well as of the sporulating cell type were found to be able to become gonidangia, as was to be expected.

3.—Formation of the symplasm and the regeneration of cells

In figures 7 to 18 on Plates B and C of our preliminary paper (*28*) the gradual dissolution of Azotobacter cells and the regeneration of various types of cells was first illustrated. Other pictures were published in Part I of these Studies (*25, Pls. XVIII to XXI*), here together with analogous findings of other authors. On Plate 9 of the present paper some additional photographs are reproduced, all pertaining to the symplastic stage of Azotobacter. Figures 21 (Pl. 2), 33 to 36 (Pl. 3), 38, 47, and 48 (Pl. 4), 56, 59, and 60 (Pl. 5), 66, 71, and 72 (Pl. 6), and 84 (Pl. 7) are also of interest in this connection.

All growth types of Azotobacter, vegetative as well as reproductive cells, were seen to enter the symplastic stage regularly. If vigorously growing vegetative cells were transferred, practically without exception

and on all substrates used the dissolution took place after 10 to 20 days, and about a week later the regeneration became general. If old material is transferred, typical symplasm or regeneration of cells may be seen, of course, from the start. If the transfers are repeated at short intervals the characteristic change between cellular and amorphous stage may occur much more frequently. For instance, transfers were made from a young culture of normal spore-free Azotobacter cells on mannite-nitrate agar and repeated each morning and each evening for several weeks. For a few days normal development took place, the next 6 to 8 transfers gave only very little dewlike transparent slimy growth made up of typical symplasm, later normal development became again visible, and so on in regular alternation. The tubes which first showed only symplasm gave normal growth after 2 to 3 days' delay, while with transfers made from vegetative cells no lag was noticeable (*25, p. 189*). Occasionally symplasm was also found in young colonies. On the other hand, cultures kept without change in mannite-nitrate solution for several years exhibited the regular alternation between symplastic and cellular stage quite clearly as long as the microscopic tests were continued.

As was described in our preliminary paper (*28*), the newly formed symplasm may be either homogeneous and not stainable with aqueous dyes or of a more or less hairy structure and easily stainable. These differences may persist until the new cells are formed, or changes from one to the other type may occur. Amoeboid motility, recorded by several authors (*25, p. 183*), was never observed, but strong inner movements in the amorphous clumps could be easily seen in the hanging drop. Encapsulated symplasm, as was described by Lankester in 1876 (*25, p. 170*), was only found in very few instances (in some, but not in all, milk cultures of the small sporulating rods). Figure 66 on Plate 6 shows three such "macroplasts," as they were called by the British author, intact but of relatively small size (most of them were twice as large as those photographed), and one which has liberated its sarcina-like mass of small globular cells, which on account of its thickness could not be properly focused in the picture. The streptococcus-like chains also visible are the next step in the development which led to a uniform coccoid growth of typical regenerative bodies. The regenerative units which first become visible in the symplasm may either gradually increase in size, as illustrated by figures 98 to 100 on Plate 9, or may agglomerate to full-sized cells (figs. 56 and 59 on Pl. 5; fig. 84 on Pl. 7; figs. 103 and 106 on Pl. 9). The regeneration of small sporulating rods is shown in figure 102; it should be compared with figure 15 on Plate C of our preliminary paper (*28*). Figure 104 illustrates the possibility of the newly-formed rods growing in a radiate arrangement, while figure 105 indicates that in other cases the new cells may appear along and parallel to the edges of the lobes of the symplasm. If the lobes are torn apart, as easily happens in making a preparate of such growth, a picture results which very closely resembles *Bacillus pediculatus* A. Koch et Hosaeus, while the appearance of the intact symplasm of this kind is very similar to *B. vermiformis* Ward and practically identical with *Newskia ramosa* Famintzin (*34, pp. 53-55, Pl. I*). There is no doubt that a thorough study of this type of bacterial development will release these species and the so-called genus Newskia from their isolated position in the system of bacteria. The bacteria visible at the edges are not the cause of the slime, as was assumed, but it is the (slimy) symplasm which produces

hese cells first at the periphery, later also in the center. The short rods visible in figure 105 became long threads, which closely followed the outlines of the lobes of the remaining slime, until this was completely converted. Irregular spirochaetoid cells, similar to those of figure 12 on Plate 1, are shown evolving from the symplastic stage in figure 107 on Plate 9. And in figure 108 queer whiplike and fungoid forms are presented which are fairly frequent in old potato cultures of the small-cell types of Azotobacter as well as of Radiobacter. They often assume much larger dimensions, in which case no clear photographic picture is obtainable and are probably identical with what was described by Winkler as "filidia" (*25, p. 175*). As, however, not always filiform but often very irregular forms are assumed by such agglomerated symplasm (fig. 21 on Pl. 2), it seems recommendable to adopt the term "sclerotia" for these comparatively solid formations. They are no "involution forms" but produce either by fragmentation or by a second passage through the symplastic stage cells of normal shape. The term "filidia," however, may be properly applied to those more or less solid large threads which often appear temporarily in the course of the regeneration of new cells from the symplasm. Figures 33 and 36 on Plate 3 illustrate this possibility, which was also discussed in Part I (*25, p. 192*).

4.—Conjunction

Conjunct cells were regularly observed in young Azotobacter cultures of large as well as of small-cell types, usually when 2 to 4 days old. In older cultures the process may repeat itself, after new cells have emerged from the symplastic stage. It is quite evident that this uniting two or more cells at the time preceding the formation of gonidia, of generative bodies, or of endospores is not without physiological significance. Especially convincing in this respect is the formation of regenerative bodies at the point where two cells united; this represents the exact counterpart to the formation of zygospores among fungi and algae. Figure 30 on Plate 3 and figure 68 on Plate 6 illustrate this occurrence very clearly.

The various modes of cell union described in Part I (*25, p. 197–203*) s characteristic of bacterial conjunction, including that of free endospores, were all observed with Azotobacter. Pictures of lateral connections and bridges, as well as the uniting of large and small cells, were demonstrated by figures 1 to 3 (Pl. A) and 22 and 23 (Pl. D) of our preliminary paper (*28*). Conjunction by bridges and by beaks are also visible in figures 278 to 294 of Part I (*25*); and figures 95 and 96 on Plate 8 of the present paper illustrate a mode of cell union which again, as the making of bridges and beaks, duplicates in a striking manner certain copulative processes known from yeasts (*25, p. 199*). That the development of endospores and of gonidangia is preceded by cell conjunction is fairly clearly indicated by the pictures shown in figures 1 (Pl. 1), 61 and 69 (Pl. 6), 81 (Pl. 7), and 89 (Pl. 8). Especially spindle-shaped and triangular gonidangia are probably always the result of the uniting of two or three cells. They are produced regularly by the large sporulating cell type as well as by the fungoid growth of Azotobacter, and they make a very interesting counterpart to analogous formations observed with spirilla. (Compare especially figure 12 on Plate 1 and figure 81 on Plate 7 of this paper with figures 86 and 87 on Plate VIII of Part I (*25*).) Undoubtedly these facts would deserve

more detailed investigation; but in view of the experimental difficulties already encountered in such studies with the comparatively large yeasts, the outlook is not very promising.

DIFFERENT TYPES OF GROWTH OF AZOTOBACTER—COMPARISON WITH OTHER BACTERIA

If the seven cell types characteristic of the life cycle of Azotobacter are separated and fully stabilized, they naturally exhibit pronounced differences in physiological and especially in cultural behavior. But our experiments revealed that there may be more than one type of growth pertaining to one type of cell form, due to different pigmentation of the bacterial growth. The large nonsporulating cells may grow white, yellowish, or brown, and the large sporulating cells either white, or yellow, or brown; but in these two cases stabilization of the differently colored strains remained more or less incomplete. On the other hand, the coccoid, the dwarfed, as well as the fungoid growths of Azotobacter, may show white, yellow-orange, or red pigmentation; and all these strains could be brought to a very marked stability. The small nonsporulating rods grew usually white, more rarely yellow; and although the small sporulating rods did not exhibit any pronounced pigmentation, they, too, showed differences of growth which thus far have been accepted as sufficient for establishing different bacterial "species." But even if only the pronounced and fairly constant differences in pigmentation are taken into account, we are confronted by the truly astonishing result that Azotobacter may present itself in not less than 14 types of growth, all so different from each other that, according to the customary methods of defining bacterial species, they all would have to be accepted as separate species belonging to five or six different genera.

Before entering upon the characterization of these different types of growth of Azotobacter a summary may be given in Table I, showing how often each type of growth was observed in our experiments, how many transformations were effected in each case, and in what direction these transformations took place. Most of the dwarfed type strains grew yellow; therefore all of them are listed in one column.

These 188 transformations effected among approximately 2,000 transfers may not appear very impressive. However, several points have to be considered in this respect in order to reach a fair valuation of these results. First, it has to be kept in mind that under the microscope the seven different cell types have been seen in all cultures, although the number of cells of different shape was frequently not large enough to permit separation by plating. Secondly, it frequently happened that when plates were made the changed cells returned to the original type of growth, or their colonies were at first so similar to the others that they escaped detection. And the third point is that the new strains, once established, repeatedly proved themselves very persistent; it was mentioned above that retransformations took place occasionally not before five or more years had elapsed, and after hundreds of transfers had been made. It might be assumed that single-cell cultures should have given better results, but as pointed out before we do not share this view.

As most of our experiments were made with *Azotobacter chroococcum* and *A. Beijerinckii*, their types of growth will always be discussed first, and then the differences obtained with regard to *A. agile* and *A. vitreum*.

TABLE I.—*Types of growth and transformations observed in strains of Azotobacter*

Type of growth at beginning of experiment.	Number of transformations to different types of growth.												
	Large spore-free cells.	Coccoids.			Dwarfed growth.	Spore-free small rods.		Fungoid growth.			Sporulating rods.		
		White.	Yellow.	Red.		White.	Yellow.	White.	Yellow.	Orange.	Small.	Large.	Total.
Large spore-free cells	....	1	2	2	4	4	1	9	0	0	5	7	35
Coccoids:													
White	3	....	1	0	3	3	0	3	0	0	3	1	17
Yellow	1	2	....	0	2	3	0	0	3	1	0	0	12
Red	1	0	0	....	0	0	0	0	0	0	0	0	1
Dwarfed growth	2	1	1	0	....	2	3	1	6	1	1	0	18
Spore-free small rods:													
White	4	5	4	0	4	....	0	1	1	1	2	1	23
Yellow	0	0	0	0	1	0	....	0	2	0	0	0	3
Fungoid growth:													
White	3	5	3	0	4	5	1	....	1	1	3	1	27
Yellow	0	0	0	0	0	0	1	0	....	0	0	0	1
Orange	0	1	0	0	1	2	0	0	1	....	0	1	6
Sporulating rods:													
Small	2	3	1	1	2	2	0	4	0	1	....	4	20
Large	5	1	4	0	4	3	0	3	0	1	4	....	25
Total	21	19	16	3	25	24	6	21	14	6	18	15	188

I.—LARGE NON-SPORULATING CELLS

In another paper, published in 1908 by the senior author together with T. Westermann (30), a detailed description was given of all peculiarities of the large Azotobacter cells, based on a comparative study of 21 strains, to which only few additions are to be made. The observations then made upon the tendency of Azotobacter to form branched threads and to grow occasionally as a yellow or as a white sarcina have now found their explanation in the discovery of the fungoid and of the coccoid types of growth. It was also pointed out in that report that in several respects, especially in its colony formation and in its growth on potato, Azotobacter may exhibit certain traits characteristic of sporulating rods. Numerous confirmative results were obtained in this direction. Not infrequently the colonies on mannite-nitrate agar did not show the typical coarse dark gray granulation, but a more or less hairy and brownish structure, indicative of the tendency to develop the rod form. The change from whitish to yellowish to brown growth was seen with both *Azotobacter chroococcum* and *A. Beijerinckii*. In 1908 it was said that probably *A. Beijerinckii* should be classed as a variety of *A. chroococcum;* our new findings prove the correctness of this view. Expecially a white strain of *A. chroococcum* (No. 10), originally isolated by Prażmowski (40), behaved exactly like *A. Beijerinckii*. And several typical strains of *A. chroococcum* kept for five years in mannite-nitrate solution assumed the same character—that is, they showed now little inclination to develop the typical dark brown to black color but produced a light yellow to light brown slimy growth. On the other hand, typical strains

of *A. Beijerinckii* were also found to be able to develop a dark brown or black color when they were kept for several months in tubes containing mannite-nitrate solution and partially immersed strips of filter paper. Directly above the solution the characteristic thick yellowish to brownish slime was always noticeable, but at the upper edge of the paper strips after four to six weeks a thin dark brown to black growth became visible, and the microscopic inspection revealed the fact that the cells in this case were much more solid and more uniformly stained than those making the more slimy light-colored growth. The latter are always granular and only partially stainable, as is characteristic of *A. Beijerinckii.* With *A. agile* analogous observations were made. The slimy growth was again of light brown color like that of *A. Beijerinckii*, and at the upper edge of the paper the same black development appeared in old cultures. If *A. agile* does not produce its characteristic green pigment, as frequently happens, its growth is hardly distinguishable from that of *A. Beijerinckii*; but here other types of growth as well as slight differences in morphology permit a clear differentiation, whereas there are no such differences between *A. Beijerinckii* and *A. chroococcum*. *A. vitreum* has never shown any pigmentation of its large nonsporulating cells, which practically always retained their typical globular shape. The only exception noticed thus far was discussed in another paper (*26*); the large rodlike forms seen in this case were inclined to assume endosporulation, which could not be fully established, however. The large globular cells of *A. vitreum* leave no doubt about their being gonidangia or microcysts, and their behavior and appearance is very similar to that of globular gonidangia and microcysts of *A. agile*. A comparison of figure 6 on Plate A of our preliminary communication (*28*) with figure 94 on Plate 8 of the present paper may illustrate this similarity. These large cells are Gram-negative, as is typical for the nonsporulating large cells of *A. chroococcum*, *A. Beijerinckii*, and *A. agile*, but the somewhat smaller globular cells of *A. vitreum*, which often occur in sarcina formation, are Gram-positive. In this as well as in all other respects they display the character of the coccoid growth common to all Azotobacter strains; in fact, with all of the latter cultures microscopic pictures were obtained, especially from potato agar, which looked exactly like *A. vitreum*. In the first Azotobacter paper (*30*) it was mentioned that a white sarcina was grown from *A. agile*, as was a yellow sarcina from *A. Beijerinckii*. No explanation could be given at that time; but to-day we know that it was the regenerative bodies we had before us, and we are now also able to show that *A. vitreum* may grow in other types equal to those of *A. agile*—that is, *A. vitreum* is a variety of *A. agile*, as is *A. Beijerinckii* of *A. chroococcum*. Cultivation of the large nonsporulating cells in slightly acid mannite-nitrate agar ($P_H6.0$) always gave during the first two weeks good typical growth with strong characteristic pigmentation; in older cultures, however, the tendency to produce small cells became very marked and was repeatedly used to great advantage. Potato agar, on the other hand, favored especially the coccoid growth, while in soil quick changes to all different cell types were observed simultaneously.

2.—COCCOID GROWTH

As was explained before, this type of growth results when regenerative bodies cease to act as such and begin to multiply by fission and by budding. It is self-evident that in the beginning such strains are often

very unstable; they either grow up to large cells or reproduce rods either by germination or by simple stretching. But if they are once definitely established (cultivation on beef or on potato agar proved best for this purpose) they may cling tenaciously to their now micrococcus-like habit of growth. White growth was most frequent (Table I, p. 415), yellow next, and pink much more rare. Changes from white to yellow and from yellow to pink were observed in some cases, in full agreement with the results recorded with Micrococci (*25, p. 31, 43*). As *A. chroococcum* as well as *A. agile* produce all three types of growth, it is easily understood why these differences in pigmentation can not be constant.

The white coccoid growth is very similar to that ascribed to *Micrococcus concentricus* Zimm. On agar smooth circular colonies are formed, which have a finely granular structure, resembling under the microscope very much those of the *Bacterium pneumoniae* Friedl. group, except that the radiate stripes are missing. On gelatine they are distinctly zoned and have lobate edges. The growth on beef agar, as well as on mannite-nitrate agar, is varying from thin dew-like to thick aerogenes-like layers. Gelatine is not or only very little liquefied. Beef broth becomes slightly turbid and has no film or ring, but heavy sediment. Milk remains unchanged, brom-cresol-purple is slightly reduced; occasionally a slow peptonization takes place, which, however, was always accompanied by a change from the coccoid to the fungoid cell type. On potato a thick, creamy yellowish grayish brownish growth, similar to that of *Bacterium aerogenes*, was observed.

The yellow coccoid growth differs from the white one practically only by the yellow pigment produced in the cells on agar, gelatine, potato, etc., and the pink growth behaves in an analogous manner, except that the growth on potato was always very scant and colorless. Gelatine liquefaction was always absent or very moderate; motility was never seen. The pink cocci gave sometimes a brown hue to gelatine as well as to potato agar. According to the customary diagnosis, these yellow and pink coccoids would have to take their places close to *Micrococcus sulfureus* Zimm. emend. Lehm. et Neum. and to *Micrococcus roseus* (Bumm) Lehm. et Neum., respectively.

Nitrogen fixation was not noticeable with these coccoid strains. That, however, occasionally positive results may be obtained is proved by the fact that a culture, once determined as *Micrococcus sulfureus* var. *tardigradus* and found to be able to assimilate free nitrogen vigorously (*27*), has shown itself to have been the vegetative growth of the regenerative bodies of a yellow large sporulating rod, which stands very close to those developed from Azotobacter.

3.—DWARFED GROWTH

As this type of growth is the result of the vegetative propagation of the gonidia, it is always at the beginning, and not infrequently for a very long time, rather inconspicuous and easily overlooked. Very small whitish circular colonies of about ½-mm. diameter, of finely granular yellow structure in the center and of white, smooth appearance at the edge, are most typical. If the growth is more vigorous, a flaky structure may become visible in the center, as with *Bacterium septicaemiae haemorrhagicae* Hueppe (*18, Table 18, VI*). It often took two weeks before the colonies became visible. Transferred to agar, the growth is again frequently very thin and dewlike, and it may remain so on all

substrates—that is, a thin, colorless slime appears on solid media, while milk is left unchanged, and in broth as well as in mannite-nitrate solution hardly any growth becomes noticeable. More frequently, however, a better development takes place after a while, and in all cases observed by us this better growth was characterized by a bright yellow coloration, with the exception of two cases where later a red pigment was produced. The microscopical aspect and the other cultural peculiarities were identical in both cases. As no such bacterial growth was fully described before, as far as we know, the necessary data may be given here.

MORPHOLOGY (PL. 3).—Typical ½ to ¾μ globular, oval, wedge-shaped, or rodlike cells, single, in short chains, or often in clumps, after two to four weeks mostly small cocci, ⅓ to ½μ. Changes to small rods, to coccoids, and especially to fungoid growth were frequently observed; occasionally also upgrowth from the symplasm to large spore-free and to large sporulating cells.

STAINING after Gram was mostly negative; only in some cases very small granules showed a positive reaction, while the rest of the cells remained negative.

MOTILITY was mostly absent, only when Gram-positive granules were present the cells developed motility by one polar flagellum.

CONJUNCTION was frequent, especially in two days old cultures on salt agar and in salt-broth. Zygospores were formed.

GONIDIA were produced after the cells had grown up to full size.

GONIDANGIA of threadlike, clubbed, or globular shape, the latter equal to normal Azotobacter cells, were produced especially on potato agar, on potato, in milk, and in mannite-nitrate solution. Thin needlelike outgrowth was seen occasionally.

REGENERATIVE BODIES of globular shape appeared, as a rule, after 2 to 3 weeks, especially on agar and on potato.

MICROCYSTS.—The small globular or oval cells often became thick-walled and germinated later to small, pale, thin-walled ovals.

ENDOSPORES.—Pale, small ovals were seen to transform themselves in toto to bright, heat-resistant bodies, which gave rise to typical sporulating rods.

SYMPLASM.—Nearly always small as well as large cells developed from the symplasm, the former always and in much greater number in the thinner portions close to the edge, the latter in smaller number in the inner thicker parts. Filidia and sclerotia were also produced occasionally, the latter resembling those shown in figure 108 on Plate 9.

COLONIES.—On beef agar and on mannite-nitrate agar circular, ½ to 1 mm. in diameter, whitish to yellowish, with finely granular to flaky yellowish structure, edge white, smooth. Occasionally larger, more slimy, and more whitish colonies were formed (changing to small rod type), or larger yellow circular colonies (changing to coccoid growth), or yellow brownish colonies with somewhat irregular edge (changing to fungoid growth). On beef gelatine small yellow, dense colonies with smooth, sharp edges were formed; gelatine was not liquefied.

AGAR SLANTS.—On beef agar and on potato agar a flat, bright yellow, especially greenish yellow, growth with somewhat irregular outlines is most frequent. Occasionally it becomes orange or yellow brownish (and the cells become in these cases fungoid). On mannite-nitrate agar the growth is thinner than on beef agar, often only dew-like and transparent. Phosphate agar gave a bright greenish yellow growth. The two red strains turned from whitish yellow to orange and later to brick red; their growth remained always very moderate.

BEEF GELATINE gave only meager, thin surface growth, transparent or yellow; the gelatine remained either firm or showed after three to four weeks a little bowl-shaped depression, but only very little liquefaction.

BEEF BROTH became slightly turbid, contained some yellowish (or reddish) sediment; but only in one case a loose, thin yellow film and a thin yellow ring were produced.

MILK remained always unchanged, except in those cases where the cells assumed fungoid growth. Some yellow (or red) sediment was always noticeable; occasionally also on the surface a ring and a few flakes of the same color appeared.

POTATO gave growth, as a rule, only after repeated inoculation. Then a little transparent slime appeared, which changed with well-growing strains to a fairly thick greenish yellow (or brick red) slimy layer. Occasionally it became dull and dry (changing to fungoid type), or remained slimy, but turned brown (changing to large spore-free cells).

MANNITE-NITRATE SOLUTION gave mostly only very little growth; it showed slight turbidity and some yellow sediment; in a few cases the solution itself became distinctly yellow.

NITROGEN FIXATION was not noticeable in pure cultures, but a very pronounced stimulating effect was exerted upon the formation of thick floating membranes in mixed cultures (with large spore-free as well as with fungoid cells).

Identical transparent and yellow strains of the dwarfed growth were isolated from *Azotobacter chroococcum*, *A. Beijerinckii*, *A. agile*, and *A. vitreum*. The two atypical cultures of red pigmentation came from one strain of *A. Beijerinckii*, while two other strains of this kind did not produce such growth. It can be safely said, therefore, that the various Azotobacter species and varieties did not exhibit any difference in their dwarfed growth. *Bacterium antityphosum* Almquist, discussed in Part I (*25*, p. *147*), is equally indistinguishable from our dwarfed Azotobacter growth morphologically as well as culturally; only the agglutination test would permit differentiation. Furthermore, one culture of *Bacillus pumilus* gave us a very similar, although very weak growth, and it is to be expected that in many other cases analogous results will be obtained, as soon as the bacterial gonidia and their behavior will be made the object of adequate studies.

4. FUNGOID GROWTH

As was discussed above, this type of growth is closely connected with the gonidial development. But whereas the gonidia, when they grow as such, become similar to a minute yellow Mycobacterium, the fungoid cells in the majority of cases grew as a white, fairly large Mycobacterium, which displays on potato a very characteristic dry, raised growth of pink color. Here the differentiation of *Azotobacter chroococcum* and *A. Beijerinckii* from *A. agile* and *A. vitreum* is very sharp. Identical strains of the white-pink mycobacterium were isolated from practically all our cultures of *A. chroococcum* and *A. Beijerinckii*, but never from *A. agile* or *A. vitreum*. A few mycobacterium cultures of yellow or orange color were branched off in both cases, but these were always closely connected with the yellow dwarfed growth common to all Azotobacter strains. According to the descriptions given by Lehmann and Neumann (*18*), Söhngen (*41*), and Vierling (*43*), our yellow cultures may be identified with *Mycobacterium luteum* Söhngen, and the orange ones with *Mycobacterium lacticola* Lehm. et Neum.; but we have also to confirm Vierling's findings that the pigmentation is not absolutely stable. Changes between yellow and orange were observed and occasionally also a temporary loss of pigmentation—that is, the same instabilities as were found with the dwarfed growth.

The white-pink fungoid growth, characteristic of *Azotobacter chroococcum* and *A. Beijerinckii*, is perhaps identical with *Mycobacterium album* Söhngen. But the description given by the Dutch author (*41*) is too short to permit a definite statement. As no other description of this type of growth seems to have been published, our notes may find a place here.

MORPHOLOGY (PL. 4).—Pale, irregular, slimy sheaths with dark granules are most common on mannite-nitrate agar and in mannite-nitrate solution. They are usually the first outgrowth of the symplasm and assume gradually more regular shape and stain more uniformly. On beef agar and in beef broth the pleomorphism typical of Mycobacterium is always noticeable. Figures VI and XII of Table 69 and Figure X of Table 70 in Lehmann's and Neumann's atlas (*18*) could have been made from our slides. In milk especially richly branched forms were seen; but continued cultivation in milk caused the transformation into slime producing small rods. On potato the inclination to assume endosporulation was very pronounced, while cultivation on potato agar gave either small nonsporulating rods or white or yellow coccoid growth. In soil and in

mannite-nitrate solution, especially with P_H 7.5, the tendency to reproduce large nonsporulating Azotobacter cells was sometimes very marked, and transformations were effected accordingly.

STAINING after Gram was always positive.

MOTILITY was always absent.

GONIDIA are often liberated in great numbers especially from the slimy sheaths (fig. 40 on Pl. 4) and were frequently seen to grow up to rods or to pass over to the yellow, dwarfed growth.

GONIDANGIA.—The large, slimy threads containing numerous gonidia may be accepted as such. In addition the large, globular cells, produced in soil and in mannite-nitrate solution, which later grew as regular Azotobacter, are typical gonidangia. Such globules, when developed from the symplastic stage on potato agar, quickly dissolved into heaps of small coccoid cells (regenerative bodies).

REGENERATIVE BODIES were produced regularly in lateral or in terminal position. The former were seen to reproduce small spore-free rods, while the latter were inclined to become endospores.

ARTHROSPORES and MICROCYSTS were frequent on all substrates, but most abundant in water.

ENDOSPORES were developed from the terminal regenerative bodies in five cases. When the microscopic picture was similar to that shown in figure 45 on Plate 4, the material was heated in beef broth or in mannite-nitrate solution first to 70° C., later to higher temperatures up to 90° C., and from successful tests cultures were made on potato and on beef agar. Heated material gave at first development intermediate between normal fungoid growth and small sporulating rods. Plating and repeated heating permitted final separation.

SYMPLASM produced very manifold growth (Figs. 38, 47, and 48 on Pl. 4, Figs. 100, 102, and 107 on Pl. 9), although the typical pale, irregular sheaths with dark granules appeared most frequent. The large spore-free cells originated always from the symplasm. Irregular sclerotia, like those reproduced in figure 108 on Plate 9, were also found with this type of growth.

COLONIES on mannite-nitrate agar and on beef agar were in the beginning smaller, but otherwise very similar to regular Azotobacter colonies. When the fungoid growth was more firmly established, the colonies appeared macroscopically whitish, flat, with raised center and irregular edge, microscopically brown in the center, of hairy structure, with thin, transparent, fringed edge, very similar to Figure X of Table 69 in Lehmann's and Neumann's atlas (*18*). When the tendency prevailed to assume a more regular rod-shaped growth, the colonies became circular with sharp edge and fine granulation, whereas the inclination to change to sporulating rods caused the appearance of smaller colonies with hairy structure and irregular moss-like edge, similar to small colonies of *Bacillus mesentericus*.

AGAR SLANTS.—A flat, dry, grayish or whitish growth with thin, irregular edge is typical on beef agar as well as on mannite-nitrate agar. Often white spots are scattered over the surface. If the small spore-free rods are developing a thicker slimy white or slightly pink growth appears, while the change to small sporulating rods is accompanied by a slightly yellowish color of the layer, which may show a few wrinkles. Potato agar produced a thick yellowish pinkish growth with yellow secondary colonies, from which a pure growth of yellow coccoids was branched off.

BEEF-GELATINE.—Irregular, thin, grayish or pinkish surface growth, little development in stab; no liquefaction.

BEEF BROTH.—Uniform turbidity, loose grayish film, whitish ring, heavy slimy white sediment.

MILK unchanged during the first days, then slowly peptonized, turning brown, becoming alkaline and very slimy; on the surface often a brittle film and a whitish ring are visible. After repeated transfers from milk to milk the peptonization and brown discoloration ceased and the milk remained neutral and became very ropy; the cells had changed from the fungoid type to small nonsporulating rods.

POTATO.—Typical raised, dry, pink growth with irregular surface and edge. Rarely a yellowish grayish brownish, more or less slimy growth was produced; in these cases the irregular cells assumed rod shape.

MANNITE-NITRATE SOLUTION became slightly acid, remained practically clear; on the surface a thin grayish film developed, which often ascended on the walls of the tubes and frequently showed the white dots characteristic of the growth of *Azotobacter Beijerinckii;* on the paper above the solution a thick slime developed, which later turned brownish, and a heavy, white, slimy sediment was formed in the solution.

NITROGEN FIXATION by pure cultures in mannite solution was not noticeable, but there was a pronounced tendency to produce the wrinkled dotted film, characteristic of crude Azotobacter cultures, especially when the fungoid cells grew in symbiosis with the dwarfed cells.

The characteristic pink growth on potato reminded the senior author that some strains of *Bacterium lactis viscosum*, tested by him in 1904 for nitrogen fixation, had presented a very similar appearance (*23*). One of these cultures was still available, as was another strain of the same organism, isolated in 1909 from slimy milk. When tested, both strains behaved exactly alike and appeared very similar to the white fungoid growth of Azotobacter. Cell morphology, colony formation, the characteristic growth in milk and on potato were the same in both cases, and it was on account of these observations that with the fungoid Azotobacter strains frequently repeated tests in milk were made which, as was mentioned above, caused indeed a transformation to slime-producing rods, which proved to be identical with *Bacterium lactis viscosum*.

5.—SMALL NONSPORULATING RODS

White and yellow nonsporulating rods were observed with all four types of Azotobacter; but their morphological and cultural behavior allowed a clear separation, those dervied from *Azotobacter chroococcum* and *A. Beijerinckii* being sharply differentiated from those of *A. agile* and *A. vitreum*.

The white slime-producing small rod form of *Azotobacter chroococcum* and *A. Beijerinckii* exhibited all marks of *Bacterium lactis viscosum* (*18, p. 316; 23, p. 587, 590*). A transfer from Adametz's original culture, which was received recently from Kral's Museum, differed considerably from the older descriptions, including that published by Adametz (*1*). The rods were of very irregular size and shape, motile by peritrichous flagella; they produced gas from mannite and various sugars, but milk was still made slimy. They also gave a yellow dwarfed strain, and a yellow as well as an orange fungoid growth identical to that of *A. chroococcum*. Furthermore, large globular and oval cells were formed quite readily which resembled typical Azotobacter cells very closely (fig. 48 on Pl. 4 and fig. 57 on Pl. 5). That *Bacterium lactis viscosum* is able to fix nitrogen was shown before by the senior author (*23*).

The yellow nonsporulating rods developed from *Azotobacter chroococcum* and *A. Beijerinckii* were practically counterparts to the white rods, as well as to the yellow strains branched off from *Bacterium coli*, *Bact. typhosum*, etc. (*18, p. 382; 25, p. 57*). On the other hand, they are directly related to the dwarfed yellow growth, as well as to the yellow fungoid type, from which they were developed and into which they could be transformed. Some strains were motile by polar flagella, others were immotile; all were Gram-negative. They liquefied beef gelatine to a varying degree, grew on beef agar as a slimy, bright yellow layer, made milk alkaline and digested it partially, and produced on potato a thick, greenish yellow to bright yellow to yellow-brownish slimy growth. The general character of the smallest rods, developed from the dwarfed growth, was sometimes very similar to that of *Bacterium turcosum* (Zimm.) Lehm. et Neum., while the larger motile or immotile yellow rods resembled more those strains described as *Bacterium ochraceum* and *Bacterium fulvum* (Zimm.) Lehm. et Neum.

The white rods observed with *Azotobacter agile* amd *A. vitreum* either produced fluorescence or left the color of the substrate unchanged In both cases, however, morphology, flagellation, reproductive organs, colony formation, and growth on the various media were those of a *Bacterium fluorescens* which did not liquefy gelatine or only weakly (*Bacterium*

putidum Lehm. et Neum.). The fluorescence, when it appeared, was mostly weak and apparently was favored by a slightly acid reaction (as is the case with the typical *A. agile*), while ammonia, contrary to the usual behavior of *Bacterium fluorescens*, remained without effect.

The yellow rod developed from the dwarfed growth of *Azotobacter agile* retained most of the cultural characteristics of its origin; merely the cell form was at least temporarily very much like that of *Bacterium putidum*, and there was a clear tendency, especially in broth cultures, to assume endosporulation, which, however, could not be fully stabilized in this case.

Quite different from the white rods of *Azotobacter chroococcum*, as well as of *A. agile*, are the two strains received from Dr. Mulvania (No. 26 and 27). Both produced large amounts of gas and displayed in every respect the cultural characters of Aerobacter (Coli-Aerogenes group). However, their tendency to make large Azotobacterlike cells was very marked, and therefore we did not feel justified to discard them lightly as "contaminations." Undoubtedly they do not belong to the life cycle of *A. chroococcum* or *A. agile;* but, as Mulvania (*36*), too, noticed gas formation in his Azotobacter cultures, there remains the possibility, or even the probability, that another species of Azotobacter exists, which should be more thoroughly studied. The upgrowth from the gonidia to large cells was very similar to that observed with other Azotobacter strains (fig. 10 on Pl. 1). The tendency to assimilate free nitrogen has been ascertained with several members of the Aerobacter group (*24, p. 688*).

6.—SMALL SPORULATING RODS

Aztobacter vitreum did not produce any sporulating rods, but 18 strains were grown from *A. chroococcum*, *A. Beijerinckii*, and *A. agile*. They again proved to be polymorphous, and equally so with both Azotobacter species. Width and length of the rods, as well as the endospore formation, varied considerably, and the cultural features proved to be equally unstable. But these alterations were by no means erratic; in fact, there was a gradual increase in the size of the rods, a progressive change from polar to central sporulation, and a simultaneous passing from weak to vigorous growth. The smallest rods with polar spores were extremely fragile and little inclined to grow on the substrates used. Frequently reinoculation became necessary, and still losses occurred. On the other hand, when left undisturbed on the same substrate they remained alive for long periods. Two of these strains first appeared in old stock cultures (*26*), and several of them survived when kept for five years in mannite solutions. When tested at the end of this period in beef broth and in mannite-nitrate solution those of *A. chroococcum* and *A. Beijerinckii* grew only in the latter, while those of *A. agile* preferred the beef broth.

Morphologically as well as culturally these small, weakly growing, Gram-negative rods with polar spores are practically identical with *Bacillus terminalis* Mig., as described by Lawrence and Ford (*10*). At first it seemed as if those of the chroococcum type could be clearly differentiated from the agile type by the appearance of the spores; the latter showing ridges similar to those of *B. asterosporus* (A. Meyer) Mig. But the cultural character was distinctly different from that of *B. asterosporus* and very similar to that of the other strains. Ford and his collaborators have seen similar ridges occasionally upon the spores of *B. brevis* Mig., *B. fusiformis* A. M. et Gottheil, and of related forms,

nd it is also our experience that this feature is rather unstable even in *B. asterosporus*, as is proved by the permanent absence of ridged star pores in the original culture, obtained from the New York Museum of Natural History.

When the strains show a somewhat more vigorous growth and their ells increase in size, the morphological and cultural characters become dentical to those of *Bacillus fusiformis* A. M. et Gottheil, according to he descriptions published by Gottheil (*12*) and by Lawrence and Ford (*10*), with the exception that not only globular spores were produced but also many of oval shape. Comparative tests made with a culture from the New York Museum gave identical results. Only on potato agar nearly all spores were globular, and these showed a pronounced inclination to become normal regenerative bodies. Apparently *B. centrosporus* Ford (*17*) should also be classed as a variety of *B. fusiformis*. The cultural marks are very similar, and the particular form of the rods is equally noticeable with otherwise typical *B. fusiformis* growth (fig. 63 on pl. 6).

The next step in the development of the small sporulating rods of *Azotobacter* chroococcum and A. *Beijerinckii* proved to be a change in cell morphology from the slender, frequently pointed forms of *Bacillus fusiformis* to a more compact appearance with rounded or square ends, centrally located spores, and a change in the cultural character to that of *Bacillus pumilus* A. M. et Gottheil. Ford was unable to obtain a typical culture of this strain, and the one we received from Kral's Museum also did not fully agree with Gottheil's description; it displayed in several respects, especially on potato, unmistakably the characters of *Bacillus fusiformis*, that is, while our cultures gaining in size and vigor of growth changed from the type of *Bacillus fusiformis* to that of *Bacillus pumilus*, the weakened stock culture of the original isolation had followed the opposite course. *Bacillus pumilus* in its typical form makes a very characteristic grayish pinkish brownish smooth growth on potato, well known to the senior author from numerous isolations made under his direction by Bierema (*6*) in 1908. Two of these cultures were still at our disposal, as was also one culture of *Bacillus Freudenreichii* (Miquel) Mig., described by the senior author in 1905 (*23, p. 719*) and later recognized as closely related to *Bacillus pumilus* (*6*). The cultural features were still fairly similar to those of the original isolations, but the spore-formation had ceased entirely, and the cells exhibited clearly a mixture of fungoid and small rod-like growth. As was to be expected from these findings further testing confirmed that this fungoid growth of *Bacillus pumilus* is identical with the white-pink Mycobacterium developed from *A. chroococcum* and *A. Beijerinckii* as well as from *Bacterium lactis viscosum*. It was not difficult to change these spore-free strains of *Bacillus pumilus* and *Bacillus Freudenreichii* by continued cultivation in milk into typical strains of *Bacterium lactis viscosum*. On the other hand we have not yet succeeded in reestablishing the endosporulation, which once was very conspicuous with these strains, whose purity and authenticity are beyond doubt. Successive transfers on potato and heating in beef broth up to 85° C. clearly favored the return to a more typical rod form (fig. 58, 54, and 46 on Plates 5 and 4) and also to the production of fairly resistant zygospores and arthrospores. But a complete success has not been reached thus far, and this experience makes it easy to understand why analogous tests with Azotobacter have been and are so often failures.

Still somewhat larger than *Bacillus pumilus* is a sporulating rod which was developed directly from the dwarfed growth (No. 2 grown

repeatedly from No. 1). A very similar strain was received from the New York Museum of Natural History as *Azotobacter chroococcum* (collection No. 522). Another subculture received from the same source under the same number in 1915 had not shown endospore formation but grew very similarly to our dwarfed Azotobacter strains. It died out after a while, and therefore we can not decide whether the sporulating growth later received from New York was a legitimate offspring or a contamination. On account of the fact that the analogous change occurred repeatedly under our eyes, the former possibility seems to us more probable. The New York strain showed all features of *B. circulans* Jordan (*17, p. 519*), and our own culture (No. 2) was fairly similar except that it was more inclined to become a large sporulating rod like those which we still have to discuss.

The small sporulating rods of *Azotobacter agile*, while resembling very closely the smallest type developed from *A. chroococcum* and *A. Beijerinckii*, did not show any tendency to pass over to the *Bacillus pumilus* type, and this is again in good agreement with the recorded absence of the white-pink fungoid growth in the cultures of *A. agile*. In cell form and colony type these sporulating rods first remained very close to those of the small spore-free rods of *A. agile* (that is, *Bacterium putidum*); later however they did not exhibit any sharp difference from the *Bacillus terminalis-fusiformis* type. They, too, could be transformed into large sporulating rods.

7. LARGE SPORULATING RODS

Here again *Azotobacter chroococcum*, *A. Beijerinckii*, and *A. agile* produced very similar growths. The strains derived from the first-named species proved to be identical, morphologically as well as culturally, with *Bacillus petasites* A. M. et Gottheil (*12*), while those obtained from *A. agile* exhibited more the character of *B. silvaticus* A. M. et Neide (*37*), but these differences are rather inconspicuous. An old stock culture of *B. petasites* received from New York was somewhat reduced in size, but otherwise quite typical. Like all strains developed from our Azotobacter cultures, it grew on beef agar either white, yellow, or brown. These three types of growth are not constant on account of the instability of the cells causing the different pigmentation. The typical, very large, broad, granulated rods produce the characteristic yellow growth; the weakly staining oval cysts, which were discussed above, make a whitish grayish layer; and in the brownish material, which also may give a brown color to the agar, sporulating long rods and threads of more or less regular shape are most frequent (fig. 73, 74, 76, and 77 on Plate 7). Temporarily, of course, one or the other mode of growth may predominate, and in short-termed tests such cultures are liable to be erroneously classified as different "species." Form, color, and structure of the colonies, although very characteristic in their typical development, may vary considerably, too. Occasionally colonies were seen which macroscopically as well as microscopically (low magnification) were indistinguishable from those of the large nonsporulating cells of *A. chroococcum;* they contained nothing but spores.

Illustrated descriptions, of two large, sporulating, nitrogen-fixing organisms (*Bacillus malabarensis*, and *B. danicus*), which were published by the senior author some years ago (*27, 29, 30*) can now be accepted as descriptions of the large sporulating cell type of Azotobacter. A renewed thorough study of our stock cultures left no doubt that they may

assume the character of this organism, as was already indicated in those earlier papers. With regard to *B. danicus* it was said in 1908 that all cell forms characteristic of Azotobacter were seen except the sarcina form; with the help of our newly won knowledge this could now be easily developed from secondary colonies on beef agar. The curious beet-shaped large cells of *B. malabarensis* can now also be identified with the gonidangia of the sporulating Azotobacter strains, and that the original isolation produced in mannite solution large globular spore-free Azotobacter-like cells (*27*) is also easily understood at present.

Bacillus luteus Baker et Smith seems to be identical with *B. petasites*, according to the detailed description published by Garbowski (*11*). The three types of growth (white, yellow, or brown), the inclination to produce in secondary colonies yellow spore-free cells (coccoids), and the various cultural marks are the same in both cases. The ability to fix free nitrogen is, of course, variable; *B. malabarensis* and *B. danicus* were found to assimilate 1 to 2 mgm. per 100 cc. 1 per cent mannite soil extract.

The potato cultures, which proved most helpful for reestablishing the spore-free typical Azotobacter growth, gave also the only opportunity to distinguish with some accuracy the sporulating strains developed from *Azotobacter chroococcum* and *A. Beijerinckii* and those of *A. agile*. For one or two weeks all of them displayed first the whitish slimy, later the yellow glossy growth characteristic of *Bacillus petasites;* but then the growth became distinctly brown when derived from *A. chroococcum* or *A. Beijerinckii*, while it turned gray with *A. agile* as with *Bacillus silvaticus*. However, a much better differentiation was secured if use was made of the conspicuous inclination of these potato cultures to give rise to other types of growth. The cultures developed from *A. chroococcum* and *A. Beijerinckii* were found inclined to resume the character of *B. pumilus*, or transferred to beef gelatine they even went back to the very characteristic white-pink fungoid growth, while those of *A. agile* returned on beef gelatine to the white, small nonsporulating nonliquefying rod closely related to *Bacterium putidum*. Still more convincing, although more difficult to attain, is of course the reestablishment of the typical nonsporulating large cells; continued culture on potato alternated with transplants to alkaline mannite-nitrate agar and solution gave comparatively the most satisfactory results in this respect.

That the large sporulating rods of the Megaterium type are much inclined to become smaller and to assume other cultural characters was often observed, and it is equally well known that stock cultures of the smaller sporulating rods not infrequently lose their ability to produce endospores and become similar to *Bacterium coli* and related forms. With the anaerobic nitrogen-fixing bacilli of the Amylobacter group these changes are even more frequent, and the ability of these organisms to produce aerobic nonsporulating coccoids brings them into closer contact with the aerobic nitrogen-fixing bacilli of the Azotobacter group. All these relations should be properly considered and more thoroughly studied.

DISCUSSION OF RESULTS

The observations recorded concerning the different types of vegetative growth and of reproduction of Azotobacter permit a much more complete and much more accurate characterization of this organism than has been given thus far to any other genus of bacteria. In fact, all form genera of

bacteria, as created by Ferdinand Cohn and his successors, have become invalid because, in accordance with earlier observations discussed in Part I (*25*), our experiments have shown that Azotobacter, like certain other natural groups of bacteria, is able to grow in all forms which were accepted as a basis for establishing the so-called genera Micrococcus, Bacterium, Pseudomonas, Bacillus, and Mycobacterium. Contrary to a very widespread, although quite illogical, assumption the pleomorphism of Azotobacter, as of all other bacteria, does not obscure but clarifies and strengthens the character of the genus as well as of its species. While a study of the large nonsporulating cells alone made it probable that *Azotobacter Beijerinckii* J. G. Lipman might be a variety of *A. chroococcum* Beij., this conclusion has been made certain by the discovery that all other types of growth are identical in both cases. *A. agile*, on the other hand, exhibits in its large nonsporulating cell form certain features which might be accepted as proof that it should be classed as another variety of *A. chroococcum*, as was done, for example, by Prazmowski (*40*); yet its small nonsporulating rod form and its fungoid growth are so different that no doubt remains about its being a true species. That *A. Vinelandii* J. G. Lipman is identical with *A. agile* Beij. could be said on account of the identity of their large nonsporulating cell forms (*30*), and all other developmental stages have now shown themselves equally identical. *A. vitreum* Löhnis, in its large nonsporulating cell type apparently quite different from the other species, has displayed in all other directions so much similarity with *A. agile* that its being a variety of this species is practically certain, although this conclusion had to be drawn from the results obtained with the only strain available.

Three other Azotobacter species have been described as *Azotobacter Woodstownii* J. G. Lipman (*22*), *A. Hilgardi* C. B. Lipman (*19*), and *A. Smyrnii* C. B. Lipman et Burgess (*20*); but they all should be canceled. The first one was never shown to be able to fix nitrogen, and its characterization is not distinct enough. The second one was only very incompletely described. According to information kindly furnished by its author it was "very similar" to *A. Smyrnii*. This like the other is no longer alive, but its illustrated description (*20*) leaves no doubt that it was identical with the sporulating large cell form of *A. chroococcum* It is true that the original description says motility and spores were lacking; but the characterization of what was accepted as vegetative cells makes it certain that only spores were seen and photographed. Every mark ascribed to *A. Smyrnii* tallies exactly with those of quickly sporulating large cells of *A. chroococcum* (Bacillus petasites).

The ability of Azotobacter to produce in certain stages of growth genuine heat-resistant endospores was accepted by the senior author (*26*) as proof that it should be classed as *Bacillus azotobacter* among the sporulating bacilli. But after our more recent studies have shown conclusively that all old form genera, including that of *Bacillus* F. Cohn emend. Hueppe, will have to be replaced gradually by natural genera, based on complete investigation of their life histories, it appeared preferable to retain and to emend the genus *Azotobacter* Beij. by adding to the large nonsporulating type of growth the six other growth types described on the preceding pages.

Contrary to our observations upon endosporulation in Azotobacter cells it was asserted by D. H. Jones (*16*), as well as by the Committee of the Society of American Bacteriologists on Characterization and Classifi-

cation of Bacterial Types (*45*) that Azotobacter does not produce heat-resistant endospores. As an analogous assertion had been made by this Committee in a preliminary report, the senior author pointed out at the Annual Meeting of the Society in 1917 that this opinion was contrary to the facts recorded by him in 1914 and 1916 (*26, 28*). The final report of the committee, however, still retains the statement that Azotobacter does not produce heat-resistant spores. Jones' assertion is in conflict with our observations and, furthermore, seems to be contradicted by his own data. He reports (*16, p. 328*) that some of his stock cultures—

produced colonies of encapsulated spore-forming rods both large and small.

Because these sporulating rods did not grow like Azotobacter, he concluded that they were contaminations. That this assumption may have been correct is of course possible, but it is equally possible that the facts observed are further proof that endosporulating rods may be produced by Azotobacter. We tried to make it clear in our first paper on this subject (*26*) that naturally the sporulating cells of Azotobacter differ in their growth as in their appearance more or less from the nonsporulating cells, but that the possibility to change them back to the original nonsporulating form proves beyond doubt that they are really a type of growth of Azotobacter and no "contamination." To assume that morphologically different cells should display the same physiological (cultural) character is, of course, quite illogical. This point was emphasized in the following statement in our preliminary paper (*28, p. 690*):

Such wide morphological differences must always be connected with no less considerable alterations of the whole physiological character, so that these other types, if they are known, of course, are stored away as entirely different "species" in various remote places in the so-called "system" of bacteria. This conclusion can be drawn with absolute certainty from our observations on *B. azotobacter* as well as from Henri's experiments with *B. anthracis*. If only those changed forms, frequently seen in all bacteriological laboratories, had not been persistently discarded as uninteresting "involution forms" or as "contaminations," the whole situation would undoubtedly be much clearer and much more satisfactory.

Transformations from and to the original type of growth alone are decisive. That they can not be expected in short-termed experiments conducted along the customary lines of bacteriological research is amply proved by the facts recorded in this and in the first part of our Studies upon the Life Cycles of the Bacteria (*25*). Negative results based upon such experiments are not conclusive.

With reference to the question of conjunction of bacterial cells, Jones (*17, p. 330*) states—

that what is here referred to as conjunction of two individual cells is rather the incomplete fission of individual cells in process of division.

That there were "side connections" which drew our attention to this point, and that these lateral bridges are clearly visible in our photographs can not be explained, however, upon the assumption of incomplete fission. That also in this case we merely rediscovered a fact which should long have found its place in the bacteriological text books was shown beyond doubt by the data we were able to collect and to present in Part I (*25, p. 197–204*).

That pathogenic bacteria—for example, the causative agents of bubonic plague, anthrax, cholera, diphtheria, etc.—are just as pleomorphous as Azotobacter, Nitrosomonas, *Bacterium radicicola*, etc., and that their

life histories follow the same fundamental lines can no longer be denied, provided that the facts recorded in the literature are taken under adequate consideration. In morphological, as well as in physiological respects, much interesting information is to be expected from such investigations.

As was frequently noticed in bacteriological tests of soils, wherein usually a normal growth of Azotobacter is to be observed, this organism may be temporarily replaced by other nitrogen-fixing bacteria, such as *Bacterium lactis viscosum* and other nonsporulating rods, various large sporulating bacilli, some micrococci, etc. The possibility that different developmental stages of the same organism may have to be made responsible for such changes sheds new light upon the very great ability of the bacteria to adapt themselves to widely varying environmental conditions. It is especially noteworthy that the life cycle of Azotobacter not only unites several of the best-known aerobic nitrogen- and nitrate-assimilating organisms, but also strong ammonia producers and forms which are known to be very able to make use of the various organic substances of the soil, first of all of the humates, namely, several mycobacteria (*41*, *43*).

SUMMARY

Tests made with 30 strains of Azotobacter and with several cultures of related bacteria have shown that only two species of Azotobacter are completely characterized thus far: *Azotobacter chroococcum* and *A. agile* Beij. (syn. *A. Vinelandii* J. G. Lipman). *A. Beijerinckii* J. G. Lipman is a variety of *A. chroococcum* and *A. vitreum* Löhnis is probably a variety of *A. agile*. *A. Smyrnii* C .B. Lipman et Burgess can not be accepted as a species; according to all marks ascribed to it by its authors it is the large sporulating growth type of *A. chroococcum*. *A. Hilgardi* C. B. Lipman and *A. Woodstownii* J. G. Lipman are both incompletely described and should not be retained.

The genus Azotobacter is characterized by the morphological and physiological behavior of its seven different cell types. These are (1) large nonsporulating globular, oval, or rodlike cells of white, yellowish, or brown color, with polar or peritrichous flagella, able to act as gonidangia and microcysts; (2) coccoid cells of white, yellow, or pink pigmentation, the vegetative growth of the regenerative bodies, identical with *Micrococcus concentricus* Zimm., *Micrococcus sulfureus* Zimm. emend. Lehm. et Neum., and with *Microcococcus roseus* (Bumm) Lehm et Neum., respectively; (3) dwarfed cell type of yellow, white, or of red color, the vegetative growth of the gonidia; (4) irregular, fungoid cells, producing a yellow, orange, or (in the case of *A. chroococcum* and *A. Beijerinckii*) a white or pink growth; the former two are closely related to the dwarfed growth and identical with *Mycobacterium luteum* Söhngen and with *Mycobacterium lacticola* Lehm. et Neum., respectively, while the latter is probably identical with *Mycobacterium album* Söhngen; (5) small nonsporulating rods of white or of yellow color, the former being identical with *Bacterium lactis viscosum* (Adametz) Lehm. et Neum. in the case of *A. chroococcum* and *A. Beijerinckii*, but with *Bacterium putidum* (Flügge) Lehm. et Neum. in the case of *A. agile* and *A. vitreum*; (6) small sporulating rods, identical with *Bacillus terminalis* Mig., *Bacillus fusiformis* A. M. et Gottheil, and in the case of *A. chroococcum* and *A. Beijerinckii* with *Bacillus pumilus* A. M. et Gottheil; (7) large sporulating cells, growing white, yellow, and brown, identical with *Bacillus luteus* Baker et Smith, *Bacillus petasites* A. M. et Gottheil, *Bacillus malabarensis*

Löhnis et Pillai and *Bacillus danicus* Löhnis et Westermann; those grown from *A. agile* showed some marks of *Bacillus silvaticus* A. M. et Neide, which, however, is closely related to *Bacillus luteus*. All cell types were transformed into each other.

The reproductive organs of Azotobacter are (1) gonidia, in part filterable, produced in small numbers, one to four, in the small cells, and in larger quantities in the large ones, which act in this case as gonidangia; (2) regenerative bodies and exospores, either produced by the cells in lateral or in terminal position, or growing up from the symplasm; (3) arthrospores, formed by fragmentation of the rod-like or fungoid cells; (4) microcysts, that is, small or large globular or oval resting cells; (5) endospores, produced singly by the rod-like cells in terminal or in central position, or to two or more in globular or spindle-shaped sporangia. Gonidia form the basis for the development of regenerative bodies, arthrospores and endospores; the production of polar regenerative bodies always preceded the establishment of endosporulation, and endospores were found to be able to reproduce gonidia and regenerative bodies.

The formation of the symplasm and the regeneration of new cells from this more or less amorphous substance of varying stainability proceeds with Azotobacter in the same manner as with all other bacteria. Amoeboid movement of symplasm was never observed, but strong inner movements were seen, and occasionally globular macrocysts were found which are analogous to the macroplasts discovered by Lankester. Besides normal cells, relatively solid agglomerations of more or less irregular shape were produced by the symplasm, so-called sclerotia, which later either transformed themselves to vegetative or reproductive cells or reentered the symplastic stage. Large "filidia," representing another type of more or less irregular and ephemeric upgrowth from the symplasm, were also observed.

Conjunction was regularly seen in young cultures before the formation of gonidia, regenerative bodies, and of exospores and endospores took place. Part of the regenerative bodies are clearly zygospores. The cell union is either temporary, effected by connecting beaks, bridges, or by direct contact of two or more cells, or permanent due either to a sticking together of two uniform cells, which retain their identity, or to a coalescence of two cells of more or less different appearance. These various modes of conjunction, observed with Azotobacter as with other bacteria, resemble very closely those recorded with yeasts and with protozoa.

The fact that the different developmental stages of Azotobacter could be in part identified with certain so-called species belonging to the form genera Micrococcus, Bacterium, Pseudomonas, Bacillus, and Mycobacterium demonstrates anew and conclusively that the whole system of bacteria needs complete revision, which is to be based upon the results of thorough examination of the life histories of the bacteria.

Some bacteriologists will be inclined to explain the phenomena reported in this paper by assuming accidental contaminations of the cultures studied. However, careful consideration of the data reported will show that most of the changes shown can not reasonably be explained upon the hypothesis of contaminations and that there are no changes reported where the reasons that might be imagined for the adoption of the contamination hypothesis deserve any more consideration than the reasons advanced for considering them to be normal changes in the life cycle of Azotobacter.

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Note.—All preparates (Plates 1-9) were stained with aqueous fuchin. Magnification in all cases X900.

PLATE 1

Large non-sporulating cells.

Fig. 1.—Large cells made up of nuclear material and slime. *Azotobacter chroococcum* (No. 24). Mannite soil extract, 2 days.

Fig. 2.—Large globular, oval, and rod-like cells, cells of medium size with nuclei. *A. chroococcum* (No. 14). Mannite-nitrate solution, 5 days.

Fig. 3.—Nuclei of cells of medium size liberated making small cells. *A. agile* (No. 16). Mannite-nitrate agar, 6 weeks.

Fig. 4.—Liberated nuclear material making regenerative bodies. *A. chroococcum* (No. 22). Mannite-nitrate solution, 7 days.

Fig. 5.—Large cells producing gonidia and regenerative bodies. *A. chroococcum* (No. 12). Beef agar, 3 weeks.

Fig. 6.—Regenerative bodies produced by large cells. *A. chroococcum* (No. 18). Beef agar, 5 days.

Fig. 7.—Large round and rod-like cells. *A. agile* (No. 16c). Beef agar, 2 days.

Fig. 8.—Typical mixture of large and medium-sized cells. *A. chroococcum* (No. 11). Mannite-nitrate agar, 1 week.

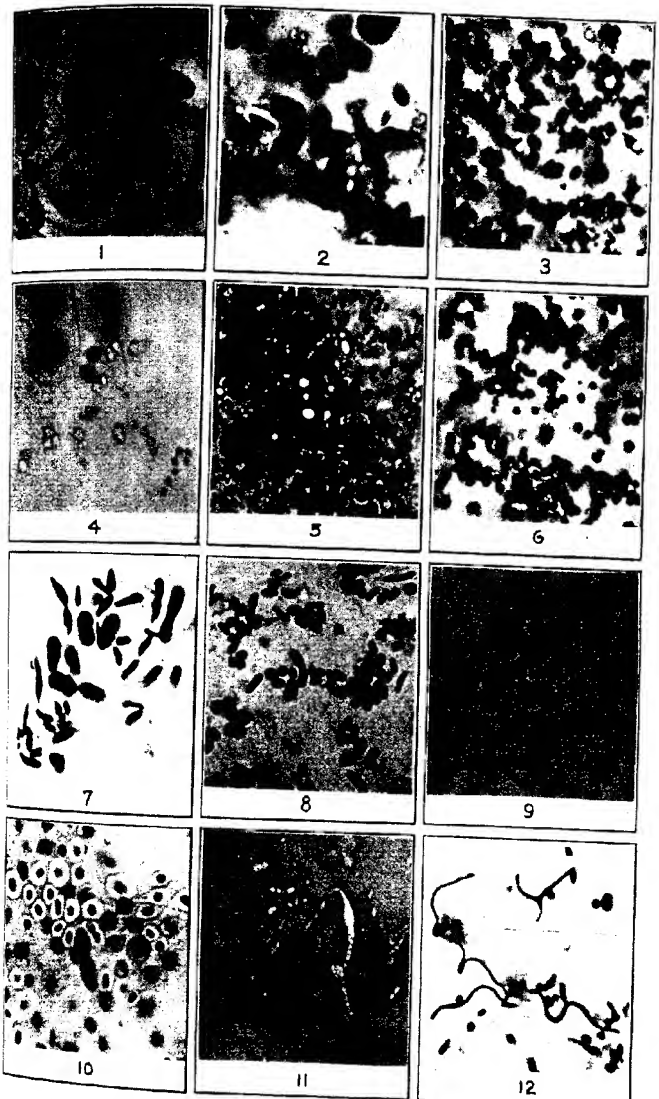
Fig. 9.—Typical mixture of medium-sized and small cells in old culture. *A. agile* (No. 16). Mannite-nitrate agar, 11 months.

Fig. 10.—Upgrowth from small to large cells. *A.* sp. (No. 26). Mannite-nitrate solution, 5 days.

Fig. 11.—Liberation of gonidia from regular and irregular cells. *A. chroococcum* (No. 22). Mannite-nitrate solution, 5 weeks.

Fig. 12.—Spirochaetoid forms, branched and budding cells. *A. Beijerinckii* (No. 13). Beef agar, 1½ month.

PLATE I



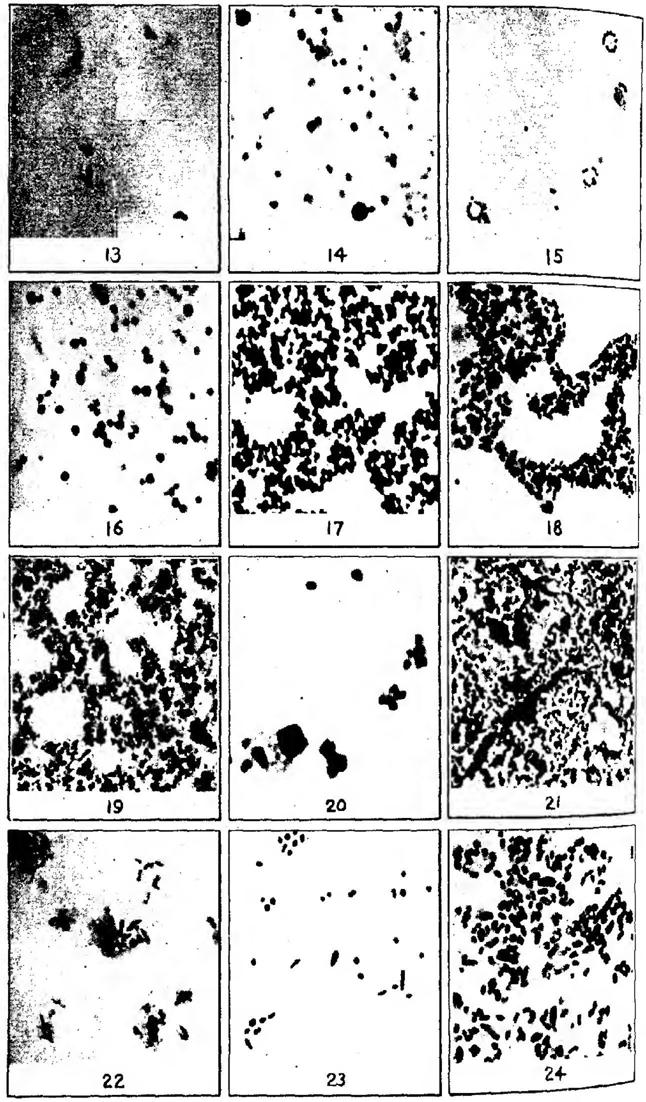

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PLATE 2

Coccoid cells.

Fig. 13.—Gonidia budding. *Azotobacter chroococcum* (No. 24). Mannite-nitrate solution, 1 day.

Fig. 14.—Gonidia growing to regenerative bodies. *A. chroococcum* (No. 18). Potato agar, 1 week.

Fig. 15.—Small regenerative bodies growing from large pale cells. *A. chroococcum* (No. 10). Mannite-nitrate agar, 6 days.

Fig. 16.—Regenerative bodies multiplying by budding and fission. *A. chroococcum* (No. 25). Mannite-nitrate solution, 2 days.

Fig. 17.—Typical coccoid growth. *A. Beijerinckii* (No. 6). Potato agar, 3 weeks.

Fig. 18.—Coccoid cells of small size. *A. chroococcum* (No. 10). Mannite-nitrate agar, 4 weeks.

Fig. 19.—Upgrowth from small to large coccoid cells. *A. chroococcum* (No. 19). Beef agar, 10 days.

Fig. 20.—Regeneration of large cells. *A. agile* (No. 7). Milk, 4 weeks.

Fig. 21.—Coccoids and sclerotia from symplasm. *Bacillus Freudenreichii* (No. 62). Beef agar, 1 week.

Fig. 22.—Coccoids reproducing small rods. *A. Beijerinckii* (No. 15). Potato agar, 5 weeks.

Fig. 23.—Coccoids reproducing small sporulating rods. *A. chroococcum* (No. 19). Mannite-nitrate solution, 5 days.

Fig. 24.—Coccoids reproducing cells of different size and shape. *A. chroococcum* (No. 25). Potato, 4 days.

PLATE 3

Dwarfed growth.

Fig. 25.—Typical gonidial growth. *Azotobacter Beijerinckei* (No. 6). Beef agar, 3 weeks.

Fig. 26.—Typical gonidial growth. *A. chroococcum* (No. 17). Mannite-nitrate agar, 5 days.

Fig. 27.—Dwarfed cells producing regenerative bodies. *A. chroococcum* (No. 1). Mannite-nitrate agar, 2 weeks.

Fig. 28.—Gonidia and slime, beginning formation of fungoid cells. *A. vitreum* (No. 9). Mannite-nitrate agar, 5 days.

Fig. 29.—Formation of fungoid cells from gonidia. *A. vitreum* (No. 9). Mannite-nitrate agar, 7 days.

Fig. 30.—Dwarfed cells assuming fungoid growth. *A. chroococcum* (No. 17). Beef agar, acid, 16 days.

Fig. 31.—Gonidia growing to small non-sporulating rods. *A. chroococcum* (No. 1). Potato agar, 7 weeks.

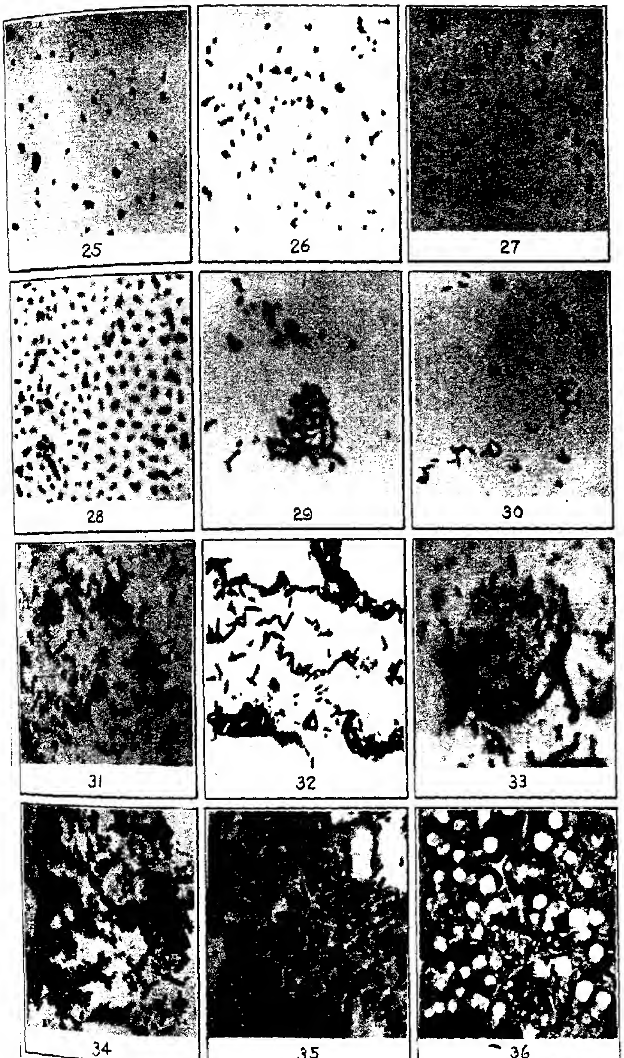
Fig. 32.—Gonidia growing to small and large rods. *A. chroococcum* (No. 1). Beef agar, 11 days.

Fig. 33.—Symplasm reproducing dwarfed growth and large slime threads (filidia). *A. chroococcum* (No. 1). Potato agar, 3 weeks.

Fig. 34.—Dwarfed growth and rods of different size from symplasm. *A. chroococcum* (No. 1). Mannite-nitrate agar, 6 days.

Fig. 35.—Upgrowth of large rods and globules from dwarfed growth. *A. chroococcum* (No. 1). Beef agar, 11 days.

Fig. 36.—Development of dwarfed cells, large globules, and branched filidia from symplasm. *A. chroococcum* (No. 1). Potato agar, 3 weeks.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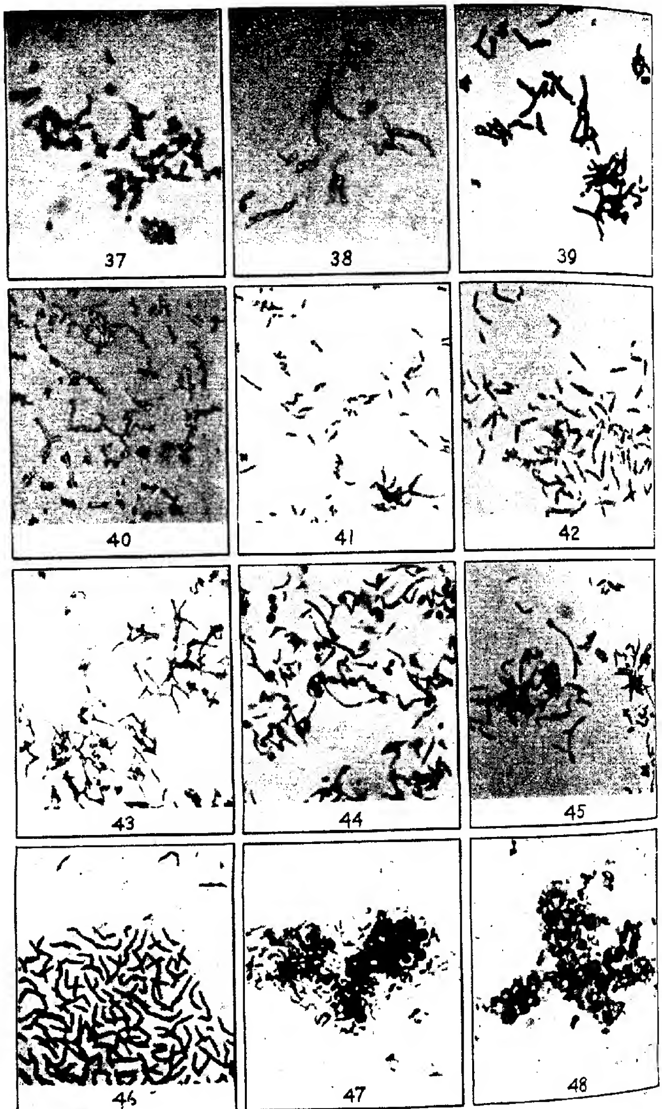
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PLATE 4

Fungoid cell type.

Fig. 37.—Typical fungoid growth. *A. Beijerinckii* (No. 15). Mannite-nitrate agar, 5 days.

Fig. 38.—Large fungoid growth. *A. Beijerinckii* (No. 15). Mannite-nitrate agar, 5 days.

Fig. 39.—Large fungoid growth, well stained. *A. chroococcum* (No. 14). Potato, 1 week.

Fig. 40.—Slime threads liberating gonidia. *A. chroococcum* (No. 17). Mannite-nitrate agar, 5 days.

Fig. 41.—Slime threads producing gonidia and small rods. *Bacterium lactis viscosum* (No. 90). Mannite-nitrate agar, 2 days.

Fig. 42.—From fungoid to coccoid and rod forms. *Bacterium lactis viscosum* (No. 90). Potato, 4 weeks.

Fig. 43.—From fungoid growth to small spore-free rods. *A. Beijerinckii* (No. 15). Beef broth, 3 weeks.

Fig. 44.—From fungoid to coccoid and sporulating growth. *A. Beijerinckii* (No. 15). Potato agar, 12 days.

Fig. 45.—From fungoid growth to spore-free and sporulating rod forms. *A. Beijerinckii* (No. 15). Beef broth, 4 weeks.

Fig. 46.—From fungoid growth to sporulating rods. *Bacillus pumilus* (No. 61). Beef broth, heated to 75° C., 1 week.

Fig. 47.—Fungoid, rod-like, and large globular cells from symplasm. *A. Beijerinckii* (No. 15). Mannite-nitrate solution, 2 weeks.

Fig. 48.—Coccoid, fungoid, and large globular cells from symplasm. *Bacterium lactis viscosum* (No. 89b). Beef agar, 1 week.

PLATE 5

Small non-sporulating rods.

Fig. 49.—Coccobacilli derived from large cells. *Azotobacter Beijerinckii* (No. 15). Mannite-nitrate agar, 7 days.

Fig. 50.—Typical coccobacilli. *A. Beijerinckii* (No. 15). Beef agar, 7 days.

Fig. 51.—Small rods with gonidia and regenerative bodies. *A. chroococcum* (No. 25). Potato, 3 weeks.

Fig. 52.—Tendency to make longer sporulating rods. *A. Beijerinckii* (No. 15). Potato, 6 days.

Fig. 53.—From small rods to fungoid and sporulating cells. *A. Beijerinckii* (No. 15). Beef broth, 4 weeks.

Fig. 54.—Tendency to return to sporulation. *Bacillus pumilus* (No. 60). Beef gelatine, 7 days.

Fig. 55.—From small rods to fungoid and globular growth. *A. vitreum* (No. 9). Salt agar, 3 days.

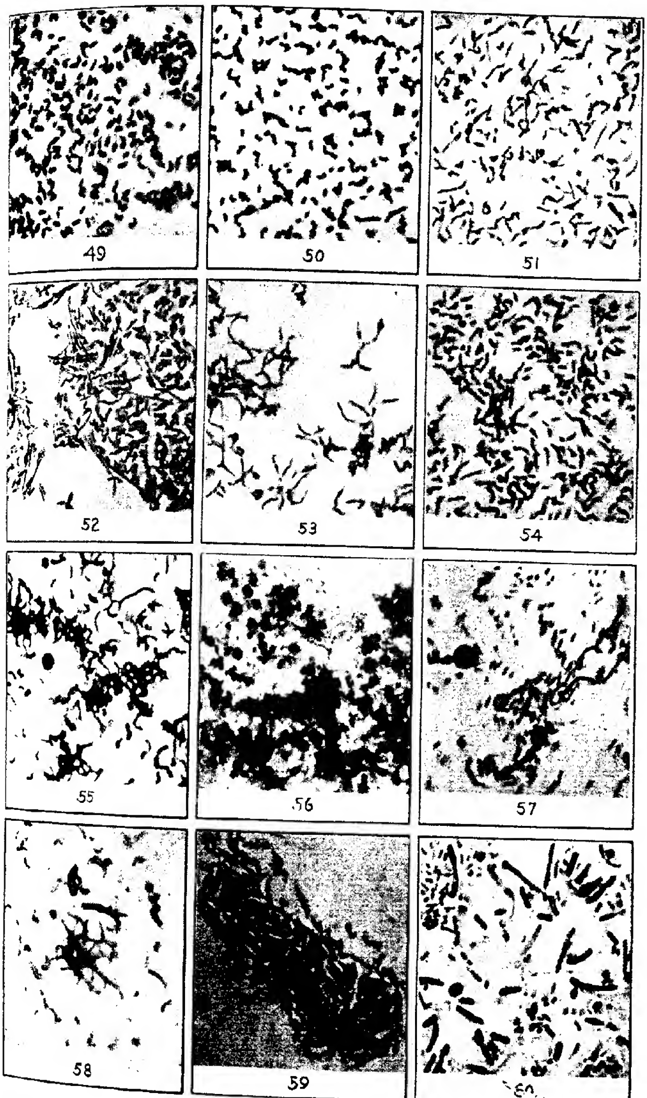
Fig. 56.—Small rods and large globules growing from symplasm. *A. agile* (No. 7c). Beef agar, 3 weeks.

Fig. 57.—Small rods producing Azotobacter-like gonidangia. *Bacterium lactis viscosum* (No. 89b). Mannite-nitrate agar, 1 week.

Fig. 58.—Small rods assuming fungoid growth. *Bacillus pumilus* (No. 61). Milk, 2 weeks.

Fig. 59.—Small and large rods growing from symplasm. *A. agile* (No. 7c). Potato agar, 1 week.

Fig. 60.—Small and large rod-like and round cells growing from symplasm. *A. agile* (No. 16b). Beef agar, 1 week.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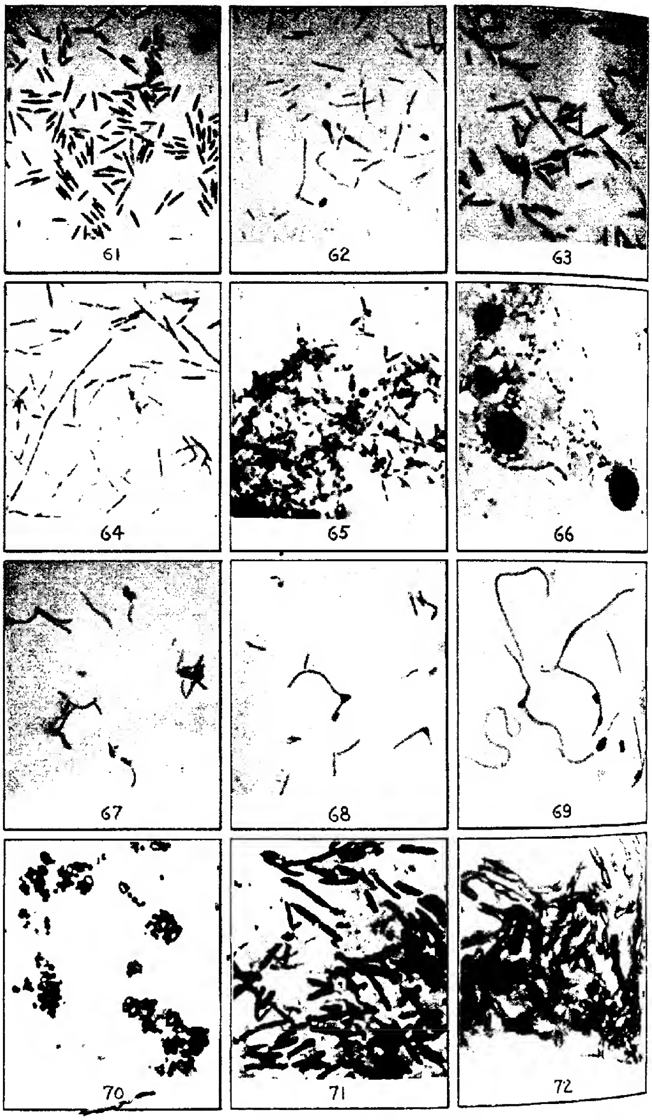
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PLATE 6

Small sporulating rods.

Fig. 61.—Typical sporulating rods. *Azotobacter Beijerinckii* (No. 5). Beef agar, 2 days.

Fig. 62.—Weakened sporulating rods. *A. agile* (No. 7). Mannite-nitrate solution, 3 weeks.

Fig. 63.—Larger sporulating rods. *A. chroococcum* (No. 19). Beef agar, 10 days.

Fig. 64.—Threads producing gonidia. *A. Beijerinckii* (No. 5). Milk, 2 months.

Fig. 65.—Threads producing gonidia, regenerative bodies, and endospores. *A. Beijerinckii* (No. 6). Beef broth, 1 week.

Fig. 66.—Arthrospore formation; encysted symplasm. *A. Beijerinckii* (No. 6). Milk, 7 weeks.

Fig. 67.—Rods producing gonidia and regenerative bodies. *A. Beijerinckii* (No. 6). Mannite-nitrate solution, 2 weeks.

Fig. 68.—Conjunction and zygospore formation. *A. Beijerinckii* (No. 6). Mannite-nitrate solution, 2 weeks.

Fig. 69.—Formation of gonidangia and regenerative bodies. *A. Beijerinckii* (No. 6). Mannite-nitrate agar, 4 weeks.

Fig. 70.—Spores producing regenerative bodies. *A. Beijerinckii* (No. 6). Beef agar, 2 weeks.

Fig. 71.—Upgrowth to large sporulating rods. *A. Beijerinckii* (No. 6). Mannite-nitrate agar, P_H 8.0, 3 weeks.

Fig. 72.—Development to large sporulating rods. *A. Beijerinckii* (No. 6). Mannite-nitrate agar, P_H 7.5, 4 weeks.

PLATE 7

Large sporulating cells.

Fig. 73.—Typical large sporulating cells. *Azotobacter Beijerinckii* (No. 3). Potato, 2 weeks.

Fig. 74.—Microcysts and vegetative rods.—*A. chroococcum* (No. 25). Beef agar, 1 week.

Fig. 75.—Branched rods and threads. *A. Beijerinckii* (No. 3). Beef agar, 12 days.

Fig. 76.—Threads with different segments. *A. Beijerinckii* (No. 4). Beef agar, 2 weeks.

Fig. 77.—Threads with gonidia and regenerative bodies. *A. Beijerinckii* (No. 3). Beef agar, 5 days.

Fig. 78.—Thread segmenting to globular cells. *A. chroococcum* (No. 18). Mannite soil extract, 3 weeks.

Fig. 79.—Globular sporangia producing 1 to 3 spores. *Bacillus danicus* (No. 34). Mannite-nitrate agar, 2 weeks.

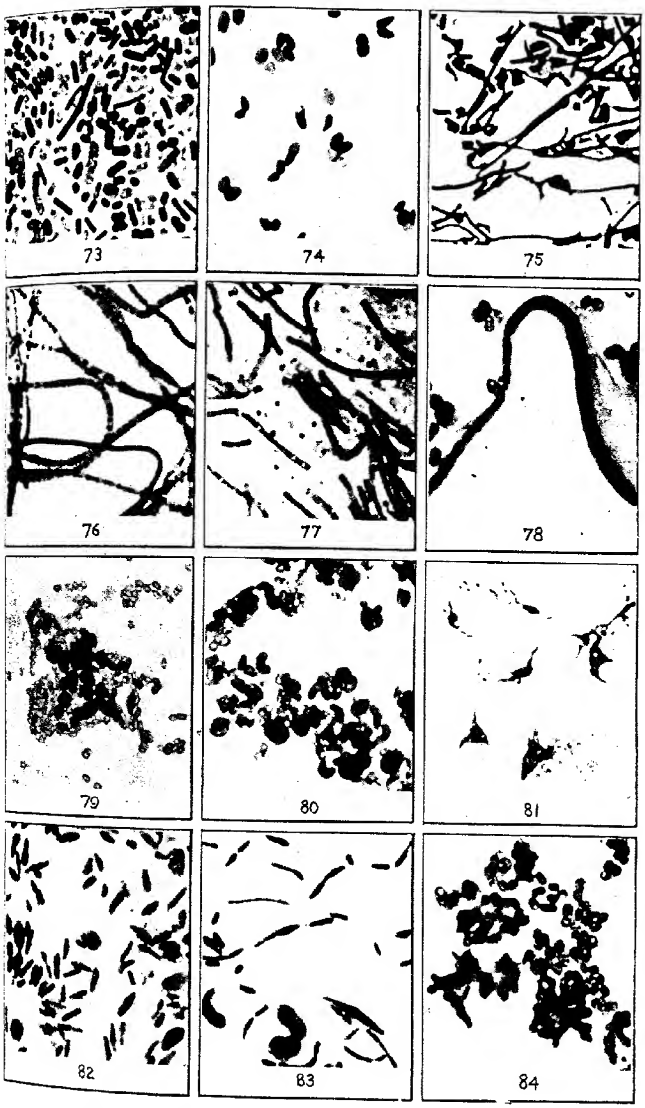
Fig. 80.—Globular cells dividing to 2 curved rods. *A. chroococcum* (No. 20). Potato, 1 week.

Fig. 81.—Branched sporangia. *A. chrococcum* (No. 25). Mannite-nitrate agar 3 weeks.

Fig. 82.—Sporulation replaced by gonidangia formation. *A. chroococcum* (No. 20) Potato, 1 week.

Fig. 83.—Beginning endosporulation of large rods. *A. agile* (No. 16c). Beef agar 3 days.

Fig. 84.—Regeneration of large globular cells from symplasm. *A. chroococcum* (No. 25). Potato, 1 week.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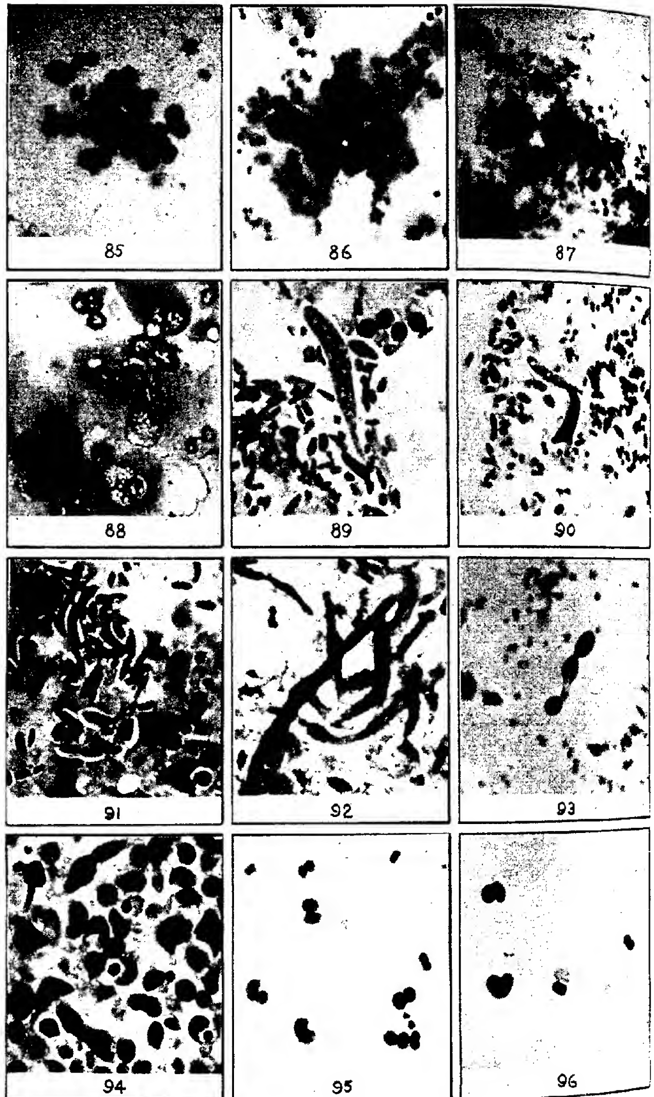
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PLATE 8

Gonidangia; conjunction.

Fig. 85.—Typical globular gonidangia. *Azotobacter chroococcum* (No. 22). Beef agar, 3 weeks.

Fig. 86.—Dissolution of gonidangia. *A. chroococcum* (No. 22). Beef agar, 1 week.

Fig. 87.—Dissolution of gonidangia. *A. Beijerinckii* (No. 4). Mannite-nitrate agar, 9 days.

Fig. 88.—Dissolution of gonidangia. *A. chroococcum* (No. 12). One percent salt-mannite-nitrate agar, 4 days.

Fig. 89.—Globular and threadlike gonidangia; also conjunction. *A. chroococcum* (No. 10). Mannite-nitrate agar, 4 days.

Fig. 90.—Regeneration of cells in gonidangium. *A. chroococcum* (No. 25). Potato, 4 days.

Fig. 91.—Globular and threadlike gonidangia; also microcysts. *A. agile*(No. 16c). Beef agar, 2 weeks.

Fig. 92.—Threadlike gonidangia producing new cells. *A. agile* (No. 16). Potato agar, 1 week.

Fig. 93.—Microcysts germinating. *A. agile* (No. 16). Mannite-nitrate agar, 2 months.

Fig. 94.—Microcysts germinating, also liberating gonidia. *A. agile* (No. 7). Potato agar, 1 week.

Fig. 95.—Conjunction and fission. *A. vitreum* (No. 9). Potato, 4 days.

Fig. 96.—Same as figure 95.

PLATE 9

Symplasm.

Fig. 97.—Formation of symplasm by regenerative bodies. *A. chroococcum* (No. 23). Potato, 9 days.

Fig. 98.—Regenerative units starting to grow. *A. chroococcum* (No. 19). Beef gelatine, 4 weeks.

Fig. 99.—Regenerative bodies growing from symplasm. *A. chroococcum* (No. 25). Beef agar, 4 weeks.

Fig. 100.—Various cell forms growing from symplasm. *A. Beijerinckii* (No. 15). Mannite soil extract, 2 months.

Fig. 101.—Branching small cells growing from symplasm. *A. chroococcum* (No. 11). Mannite-nitrate solution, 5 days.

Fig. 102.—Sporulating small rods growing from symplasm. *A. Beijerinckii* (No. 15). Mannite-nitrate solution, 17 days.

Fig. 103.—Beginning formation of rods. *A. vitreum* (No. 9). Mannite-nitrate solution, 10 days.

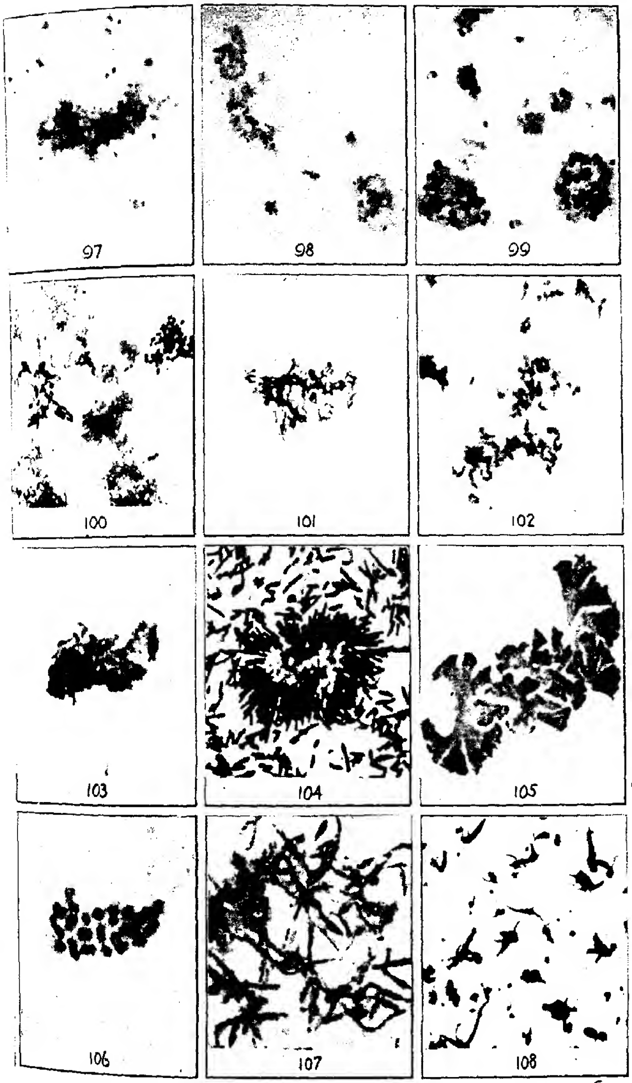
Fig. 104.—Radial growth of rods from symplasm. *Bacillus fusiformis* Gottheil (No. 149). Beef agar, 2 weeks.

Fig. 105.—Tangential growth of rods from symplasm. *A. Beijerinckii* (No. 6). Mannite-nitrate solution, 2 weeks.

Fig. 106.—Formation of new cells by agglomeration of regenerative units. *A. chroococcum* (No. 22). Mannite soil extract, 4 days.

Fig. 107.—Formation of filidia by agglomeration. *A. Beijerinckii* (No. 15). Mannite soil extract, 8 months.

Fig. 108.—Formation of sclerotia. *A chroococcum* (No. 19). Potato, 4 weeks.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INFLUENCE OF FERTILIZERS CONTAINING BORAX ON THE GROWTH AND FRUITING OF COTTON[1]

By J. J. SKINNER, *Biochemist, Soil-Fertility Investigations, Bureau of Plant Industry,* and F. E. ALLISON, *Soil Biochemist, Fixed Nitrogen Research Laboratory, United States Department of Agriculture*

The injury to cotton and other crops during the season of 1919 by fertilizers containing borax led to considerable experimentation, and a number of reports have been made recording the results of these studies on the effect of borax and fertilizers containing borax on crops, especially on corn, cotton, and potatoes. The extent of the injury to cotton and potatoes in 1919 is described by Schreiner, Brown, Skinner, and Shapovalov (*8*)[2] in a report published in 1920. Other reports on the injury to cotton have been made by Blackwell and Collings (*1*) and Plummer and Wolf (*7*). Conner (*4*, *5*) first investigated the effect of borax on plant growth; his work was with corn, although later he also studied its effects on several other plants. Morse has reported upon the effects of borax in fertilizer in Maine (*6*).

In order to study the matter fully, a series of field experiments planned by the Office of Soil-Fertility Investigations of the Bureau of Plant Industry, United States Department of Agriculture, was conducted in 1920 on several types of soil and on several crops. Arrangements were made to conduct these experiments at Presque Isle, Me., cooperatively with the Maine Agricultural Experiment Station; at New Brunswick, N. J., cooperatively with the New Jersey Agricultural Experiment Station; at Muscle Shoals, Ala., cooperatively with the Fixed Nitrogen Research Laboratory, then of the War Department; and at the Arlington Experimental Farm, Va.

Potatoes were grown in Maine, potatoes and corn in New Jersey, cotton and corn in Alabama, and potatoes, corn, Lima beans, snap beans, and cotton at the Arlington Experimental Farm. The results obtained with potatoes and corn in New Jersey by Blair and Brown (*2*) have been given in a previous publication and a report of the work conducted by Brown (*3*) with potatoes in Maine has recently appeared.

In the present article the results obtained with cotton at Arlington Experimental Farm, Va., and at Muscle Shoals, Ala., are presented. The plan of the experiment involved the use of a fertilizer analyzing 4 per cent NH_3, 8 per cent P_2O_5, and 4 per cent K_2O, which was applied to cotton at the rate of 1,000 pounds per acre. This fertilizer was made from acid phosphate, muriate of potash, sodium nitrate, ammonium sulphate, and cottonseed meal. The fertilizer free from borax served as a control. Borax was mixed with this fertilizer in such proportions as to make the applications of anhydrous borax 5, 10, and 20

[1] Accepted for publication Aug. 24, 1921.
[2] Reference is made by number (italic) to "Literature cited," p. 443.

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pounds per acre. In order to determine the effect of the rainfall and weather conditions on the action of the borax, the experiment was repeated at both places several times. The first application and planting were made early in June, and again at intervals of about a week. Six of these tests were made at Arlington and six at Muscle Shoals. It was realized that cotton planted at as late a date as this would not fully mature in either locality, but it was considered desirable to have some information concerning the action of borax on this crop, although the season was already advanced. The injury by borax has been shown to be principally to the young plant and in the early period of its growth. The experiments were therefore undertaken, and the effects on germination, on growth, and on boll formation were noted and recorded.

RESULTS OBTAINED AT ARLINGTON, VA.

The experiments conducted on the Arlington Experimental Farm were on a rich, silty loam soil, admirably suited for truck and general farm crops. The land is level and uniform and is tile drained.

The plan of the experiments involved the application of the fertilizers in the seed drill and by broadcasting; also the planting of the seed immediately after applying the fertilizers as well as after an interval of a week. A single row 132 feet long was used for each treatment. To one-third of the row, 44 feet, the fertilizer was applied in the drill and the seed was planted one week later. This is shown in section 1 of Table I. In section 2, which is the second 44-foot length, the fertilizers were applied in the drill as in section 1, and the seed was planted immediately. On the third section of the row the fertilizers were sown broadcast and the seed was planted immediately. Where the fertilizer was put in the drill it was mixed with the soil by raking with a hoe and was covered with an inch or two of dirt before the seeds were planted. In the broadcasted section the area was raked to mix the fertilizers. Each treatment, or plot, covered an area of 1/270 acre. The cotton was thinned to 45 hills per plot and two plants in each hill. Fertilizer analyzing 4 per cent NH_3, 8 per cent P_2O_5, and 4 per cent K_2O, was used and applied at the rate of 1,000 pounds per acre. In one row the fertilizer without borax was used as a control; in the second row the fertilizer containing borax in sufficient quantity to apply 5 pounds of anhydrous borax per acre was used; and in the third row the fertilizer contained sufficient borax to add 10 pounds per acre. In some of the tests 20 pounds of borax per acre were used. In order to determine the influence of weather conditions on the effect of borax on cotton, the experiments were repeated six times; the first series (A) was begun on June 2, the second (B) on June 9, the third (C) on June 18, the fourth (D) on July 7, the fifth (E) on July 15, and the sixth (F) on August 3. Notes were taken during the summer, and the plants in each plot were measured for height when several weeks old. This was done by measuring a large number of plants and taking the average. A record of the number of bolls and squares that formed was made. The plants were cut on October 1 and the green weights were taken and recorded. The complete data are given in Table I.

TABLE I.—*Effect of borax in fertilizers on the growth and fruiting of cotton grown in a productive silty loam soil at Arlington farm, Va., in 1920* [a]

Experiment series, date of fertilizer application, and quantity of borax applied per acre.	Fertilizers applied. Sec. 1.—In drill and seed planted one week later. Height of plants.	Weight of green plants.	Increase or decrease.	Bolls and squares.	Increase or decrease.	Sec. 2.—In drill and seed planted immediately. Height of plants.	Weight of green plants.	Increase or decrease.	Bolls and squares.	Increase or decrease.	Sec. 3.—Broadcast and seed planted immediately. Height of plants.	Weight of green plants.	Increase or decrease.	Bolls and squares.	Increase or decrease.
	Inches.	*Pounds.*	*Per cent.*	*Number.*	*Per cent.*	*Inches.*	*Pounds.*	*Per cent.*	*Number.*	*Per cent.*	*Inches.*	*Pounds.*	*Per cent.*	*Number.*	*Per cent.*
Series A, June 2:															
No borax	16.9	61	....	1,680	....	12.5	60	....	1,777	....	14.8	73	....	1,435	....
5 pounds	12.4	56	−8.2	1,295	−22.9	11.6	59	−1.7	1,000	−43.7	14.1	80	+9.6	1,252	−12.7
10 pounds	11.2	58	−5	1,315	−21.7	10.1	61	+1.7	967	−45.6	11.0	55	−24.7	901	−37.2
Series B, June 9:															
No borax	8.8	67	....	1,478	....	11.5	72	....	1,441	....	13.0	72	....	1,525	....
5 pounds	8.1	69	+3	1,400	−5.3	7.3	62	−14	1,305	−9.4	10.0	79	+9.7	1,503	−1.4
10 pounds	6.9	67	0	1,302	−11.9	6.5	58	−19.5	1,361	−5.6	8.8	68	−5.6	1,600	+5
Series C, June 18:															
No borax	9.6	62	....	1,500	....	10.2	69	....	1,697	....	10.7	68	....	1,692	....
5 pounds	7.0	62	0	1,590	+6	9.6	70	+1.4	1,750	+3.1	9.1	71	+4.4	1,800	+6.4
10 pounds	6.7	57	−8	1,490	−.7	8.7	72	+4.4	1,505	−11.3	7.8	64	−5.9	1,613	−4.7
20 pounds	6.5	56	−9.7	1,380	−8	6.8	49	−29	1,480	−12.8	7.2	53	−22	1,520	−10.2
Series D, July 7:															
No borax	....	40	....	1,100	....	....	45	....	1,539	....	....	54	....	1,610	....
5 pounds	....	37	−7.5	1,000	−9.1	....	49	+8.9	1,392	−9.5	....	51	−5.6	1,630	+1.2
10 pounds	....	29	−27.7	760	−30.9	....	33	−26.7	1,050	−31.7	....	41	−24.2	1,210	−24.8
20 pounds	....	21	−47.5	597	−45.7	....	29	−35.5	930	−39.5	....	35	−35.2	1,030	−36
Series E, July 15:															
No borax	....	26	....	622	....	....	28	....	823	....	....	35	....	1,146	....
5 pounds	....	26	0	680	+9	....	24	−14.3	822	−.1	....	36	−2.9	1,137	−0.8
10 pounds	....	21	−19.2	585	−6	....	23	−18	718	−12.7	....	33	−5.8	1,005	−12.3
20 pounds	....	21	−19.2	560	−10	....	18	−36	434	−47.2	....	30	−14.3	1,020	−11
Series F, August 3:															
No borax	....	10	....	0	....	....	13	....	0	....	....	17	....	0	....
5 pounds	....	10	0	0	....	....	11	−15.4	0	....	....	14	−17.6	0	....
10 pounds	....	8	−20	0	....	....	12	−7.7	0	....	....	14	−17.6	0	....
20 pounds	....	9	−10	0	....	....	8	−38.4	0	....	....	13	−23.5	0	....

[a] The measurements were made in experiments A and B, sections 2 and 3, on July 27; in section 1, August 3; in experiment C, sections 2 and 3, on August 3; and in section 1, on August 10.

An examination of the data given in Table I shows generally that the growth of the cotton was checked and the fruiting decreased by the borax. The degree of injury, however, varies with the different plantings and with the different methods of applying the fertilizers. The germination was rather irregular where 20 pounds of borax were applied, and when the cotton was young some died in spots. Ten pounds had a decided effect on the color of the foliage in each experiment. Where borax was used the foliage was much lighter green.

EFFECT UNDER DIFFERENT METHODS OF APPLYING FERTILIZERS

In section 3, where the fertilizers were sown broadcast and 5 pounds of borax applied per acre, no injury was observed in series A, B, and C, and only a slight reduction in growth in series D, E, and F. There was only a slight reduction in the fruiting of the plants. With 10 pounds of borax there was a decided decrease in growth and in the number of bolls formed. The degree of harmfulness produced by borax in the different plantings is noticeable. It is probably due to weather conditions to be considered later. Where 20 pounds of borax were used there was a decided harmful effect. In series C, D, E, and F there was a reduction in growth of 22, 35.2, 14.3, and 23.5 per cent, respectively, and a reduction in boll formation of 10.2, 36, and 11 per cent in series C, D, and E, respectively. In section 2, where the fertilizers were applied in the drill and the seed was planted immediately afterwards, the harmfulness of borax when 10 or 20 pounds per acre were used was marked. The growth was checked more in section 2 than where the fertilizers were sown broadcast. Five pounds per acre reduced growth considerably in series E and F. In section 1, where the planting was not made until one week after the fertilizers were applied and where in each case a rain intervened, the harmfulness of the borax is considerably less than in sections 2 and 3, except in series D and E. To judge from the data as a whole, it can hardly be concluded that 10 pounds were harmful when applied as in this section of the experiments.

A record of the height of the plants made when the crop was young shows that the growth was checked in the very beginning by the borax. In plate 1, A, is shown the effect of borax on the young plants in series A. In the foreground the seed was planted in the drill immediately after applying the fertilizer, and in the back half of the row the fertilizers were sown broadcast. The broken stand is readily discernible in the row receiving 10 pounds of borax. The cotton had not been thinned to a stand when the photograph was made. The young plants died in spots soon after emerging to the surface. In plate 1, B, the surviving plants are shown after having made considerable growth. It is seen here that the 10-pound borax row is much smaller than the control and the 5-pound borox row. In plate 2, A, is shown the cotton in series D. Here the broken stand in the last row, which has 20 pounds of borax, is shown.

INFLUENCE OF RAINFALL

In order to study the influence of rainfall on the effect of borax on the crop, the weekly record of the rainfall together with the maximum and minimum temperature at the Arlington Experimental Farm during the period of the experiments is given in Table II.

TABLE II.—*Temperature and rainfall at Arlington Farm, Va., June to September, 1920*

Week of—	Rainfall.	Temperature.		Week of—	Rainfall.	Temperature.	
		Maximum.	Minimum.			Maximum	Minimum.
	Inches.	°*F.*	°*F.*		*Inches.*	°*F.*	°*F.*
May 30 to June 5	1.19	90	47	Aug. 1 to 7	0.71	87	53
June 6 to 12	1.08	95	51	Aug. 8 to 14	1.13	92	66
June 13 to 19	.36	93	57	Aug. 15 to 21	2.57	91	66
June 20 to 26	1.68	84	56	Aug. 22 to 28	.29	85	62
June 27 to July 3	1.96	94	57	Aug. 29 to Sept. 4	.11	90	48
July 4 to 10	.62	89	55	Sept. 5 to 11	2.01	84	52
July 11 to 17	.41	91	63	Sept. 12 to 18	.01	87	45
July 18 to 24	1.19	95	59	Sept. 19 to 25	.03	90	44
July 25 to 31	.80	92	53	Sept. 26 to Oct. 2	1.43	88	55

Comparing the rainfall record at or about the time the fertilizers were pplied in the various experiments, it is seen that the moisture conditions ere about optimum when and after the plantings were made in series , B, and C. The rainfall, however, was very light during the weeks f July 4 and July 11 and was again light the weeks of July 25 and Auust 1. The effect of the borax in experiments D and E, which were lanted in the period of dry weather, is more severe than in the experiments which were planted when the moisture was normal. For example, n section 1, 20 pounds of borax per acre reduced the growth 9.7 per cent n series C, 47.5 per cent in series D, and 19.2 per cent in series E. In ection 2, the growth was reduced in series C 29 per cent; in D 35.5 per ent, and in E 36 per cent. In section 3, the growth was reduced 22 er cent in series C, 35.2 per cent in D, and 14.3 per cent in E. The ormation of bolls was also reduced more in series D and E than in C. A few days after the plantings were made in the latter experiments a ight rain fell, which was followed by a dry period. While the plants vere young in the earlier experiments there were occasional heavy rains, and at no time did the soil become very dry. It is not probable that a rainfall of 1.08 to 1.68 inches in one week distributed over a period of several days would wash very much borax out of the reach of the roots of the cotton. However, it would result in the diffusion of the borax through the soil, and this diffusion could undoubtedly account for the lesser degree of injury in series A, B, and C. Under the rainfall conditions of series D and E the borax was concentrated in locations surrounding the roots of the young plants and would naturally cause a more severe injury and a greater retardation of growth.

The data as a whole show that the effect of borax on cotton under the weather conditions prevailing at the time of this test is decidedly harmful when 20 pounds per acre are applied in the drill or sown broadcast. This quantity showed harmful effects whether the seed was planted immediately after the fertilizers were applied or after the intervening of a light rain. When applied in the drill, 10 pounds per acre checked growth decidedly, but were only slightly harmful when sown broadcast.

RESULTS OBTAINED AT MUSCLE SHOALS, ALA.

PLANTINGS ON CLARKVILLE LOAM

The experiments at Muscle Shoals were made on soil of Clarkville loam, located on a gentle slope, and well drained. The soil is fairly retentive of moisture and does not become compact. The plan of the experiments differed somewhat from that at the Arlington Experimental Farm in that the fertilizer was applied only in the drill and the seed was planted immediately as in section 2 of the Arlington tests. The 4-8-4 fertilizer was used at the same rate of application (1,000 pounds), and borax was used so as to apply 5, 10, and 20 pounds of anhydrous borax per acre. Two controls in which no fertilizer was used were added. One row 100 feet long was used for each treatment, which makes each plot approximately 1/110 acre. The test was repeated six times. The first was started June 12 and the others followed at intervals of about one week. The separate plantings are designated as series A, B, C, D, E, and F. Cleveland Big Boll cotton was used.

In Table III are given the data for this set of plots, including the height of the cotton plants at intervals during growth, the number of bolls which formed, and the green and dry weights of the plants, including the roots.

TABLE III.—*Effect of borax in fertilizers on the growth of cotton, on Clarkville loam at Muscle Shoals, Ala., in 1920*

Experiment series, date of starting, and quantity of borax applied per acre.	Average height of plants. July 28.	Average height of plants. Aug. 20.	Average height of plants. Oct. 18.	Bolls on Oct. 26.	Green weight of plants.	Increase or decrease.	Dry weight of plants.
	Inches.	*Inches.*	*Inches.*	*Number.*	*Pounds.*	*Per cent.*	*Pounds.*
Series A, June 12:							
No fertilizer	....	12.3	28.8	237	31	....	10
No borax	12.9	25.3	45.5	667	93	....	36
5 pounds	9.6	21.8	44.4	583	86	− 7.6	33½
10 pounds	7.1	17.3	42.1	456	85	− 9.4	32
20 pounds	4.9	13.2	32.6	242	44	−52.7	15
Series B, June 19:							
No borax	12.0	28.6	50.0	716	104	....	40
5 pounds	10.5	25.7	43.3	563	84	−19.2	34½
10 pounds	8.0	24.1	43.0	576	78	−25.0	31½
20 pounds	6.9	15.6	39.1	383	63	−39.4	25
Series C, June 26:							
No borax	9.4	18.1	45.2	337	86	....	34
5 pounds	6.3	15.1	40.5	220	78	− 9.3	28½
10 pounds	6.9	12.9	39.9	224	75	−12.8	26
20 pounds	5.0	9.6	31.5	97	43	−50.0	15
Series D, July 3:							
No borax	6.2	16.1	42.2	112	63	....	25½
5 pounds	5.2	15.1	41.6	94	62	− 1.6	24
10 pounds	4.1	12.0	32.9	92	59	− 6.4	23
20 pounds	3.2	7.2	29.3	53	39	−38.1	13
Series E, July 11:							
No borax	....	12.3	36.8	45	55	....	17½
5 pounds	....	11.5	33.7	48	56½	+ 2.7	20
10 pounds	....	9.9	31.3	46	50½	− 8.2	20
20 pounds	....	8.4	26.1	14	38	−31.0	11
Series F, July 20:							
No fertilizer	....	5.1	9.5	0	5½	....	1
No borax	....	10.0	28.2	5	46½	....	15
5 pounds	....	8.9	29.1	7	47	+ 1.0	15
10 pounds	....	8.7	25.7	4	41	−11.9	11
20 pounds	....	7.6	22.4	1	35	−24.8	8

Table IV records the weekly rainfall and temperatures at Florence, Ala., 2 miles from United States Nitrate Plant No. 2, so that the relation of rainfall to the degree of harmfulness of borax in the different series of the Muscle Shoals experiments may be correlated.

TABLE IV.—*Temperature and rainfall at Florence, Ala., June to September, 1920*

Week of—	Rainfall.	Temperature.		Week of—	Rainfall.	Temperature.	
		Maximum.	Minimum.			Maximum.	Minimum.
	Inches.	°*F.*	°*F.*		*Inches.*	°*F.*	°*F.*
June 6 to 12	0	97	53	August 1 to 7	0	94	60
June 13 to 19	.52	98	63	August 8 to 14	5.10	90	66
June 20 to 26	.58	92	56	August 15 to 21	4.46	91	64
June 27 to July 3	.08	93	61	August 22 to 28	.59	91	56
July 4 to 10	.76	94	62	August 29 to September 4	.42	96	61
July 11 to 17	.98	93	61	September 5 to 11	2.17	93	55
July 18 to 24	1.50	98	63	September 12 to 18	.32	93	55
July 25 to 31	.06	95	57	September 19 to 25	.54	92	55
				September 26 to October 2	0	92	37

From the results of series A it will be observed that 5 pounds of borax per acre produced some injury to cotton when applied in the row. Larger quantities showed even greater toxic effects. From the figures showing the average height of plants it will be seen that the injury was the greatest when the plants were small. A marked recovery followed, but the initial retarding was not entirely overcome. The green weight of plants was decreased 7.6 per cent by 5 pounds of borax per acre and 52.7 per cent by 20 pounds. The boll formation was decreased in about the same proportion or to an even greater extent, and in addition maturity was delayed. The cotton in series A was planted when the soil was too dry to germinate the seed. On June 18 a light shower fell, approximately 0.34 of an inch, which was sufficient to cause germination, and for three weeks following but little rain fell. As seen in Table IV, 0.52 of an inch of rain fell the week of June 13, 0.58 of an inch the week of June 20, and 0.08 of an inch the week of June 27. The season for the three weeks following the planting of the cotton in series A was rather dry.

The cotton in series B was planted just subsequent to a light shower and germinated in minimum time. The rainfall during the early period of growth was rather light. In the first week after planting 0.58 of an inch of rain fell, the second week 0.08 of an inch, and the third week 0.76 inch. The growth was about the same as in series A, except that in series B, 5 pounds of borax caused a relatively greater injury and 20 pounds less injury than in series A. The same general tendency prevailed, however, and the same visible effects on the young plants were in evidence. The retarding effects of the borax continued throughout the growing season.

At the time of planting series C the soil was very moist, but for more than a week after planting no rain fell and the soil became very dry. In

the second week after planting 0.76 of an inch of rain fell, which thoroughly moistened the ground. The amount of injury produced by the different quantities of borax corresponded very closely to the results in series B.

The planting of series D was made when the soil was very dry, and the seed did not germinate immediately. A good rain occurred about one week later, and about 0.76 of an inch fell; the following week there was 0.98 of an inch precipitation, and the third week 1.5 inches. After the germination of the seed there was more rainfall, and the soil was consequently more moist than when the cotton was young in the plots planted earlier. The injuries attributable to the borax were scarcely as extensive in this series as on the plots planted earlier, but they were in the same order. In series D 5 pounds of borax reduced the green weight 1.6 per cent, 10 pounds 6.4 per cent, and 20 pounds 38.1 per cent. In series C the reduction was, respectively, 9.3, 12,8, and 50 per cent; in series B, 19.2, 25, and 39.4 per cent; and in A, 7.6, 9.4, and 52.7 per cent.

In series E the cotton was planted when the soil was moist and in good tilth. Several days after planting 0.98 of an inch of rain fell, which was followed by a period of one week without rain. This period was followed by several days of rain, and, as seen in Table IV, during the week beginning July 18 1.5 inches of rain fell. The toxic effects were not as marked as on the plots planted earlier. There was a slight stimulation where 5 pounds of borax per acre were used, 10 pounds reduced the growth 2.7 per cent, and 20 pounds 31 per cent.

Series F was planted very late, and growth continued only about two months. The data are given in the table, however, and it will be seen that the effects of the borax were of about the same magnitude as in series E.

In Plate 3 the effect of borax is shown on the cotton in series A, planted June 12. The views shown at A, B, and C were photographed on July 29, August 8, and September 18, respectively, the last date being shortly before the plants were cut and weighed. Plate 3, A, was photographed before the cotton was thinned; the unbroken stand and uneven appearance of the row receiving 20 pounds of borax per acre is shown.

In Plate 4, A and B, are shown the effects of borax in series D. View A of Plate 4 was photographed on August 23 and B on September 18. In Plate 4C, the cotton in series F is shown.

PLANTINGS ON COLBERT SILT LOAM

Other experiments with cotton were begun at Muscle Shoals, Ala., similar to those at Arlington Farm, Va., and to those in New Jersey with potatoes and corn. These included immediate planting of seed after applying fertilizer, delayed planting, and broadcasting. The quantities of borax used varied from 1 to 400 pounds per acre. Two rows 70 feet long were used for each treatment on the Colbert silt loam, which is a heavier soil than the Clarkville loam. The fertilizers were applied on May 10. The rainfall for the month prior to starting the experiments, and for a like period afterwards, was exceedingly heavy. The soil became very compact from the excessive rains, resulting in a very poor stand over the entire area. The experiments were continued, however, in order to observe the effects of the borax; but a harvest was not made, as the broken stand appeared to make it useless.

Borax caused the greatest injury to cotton in the early stages, either preventing germination or in lesser amounts merely retarding growth and preventing chlorophyll formation. A record of observations made three weeks after planting is given in Table V.

TABLE V.—*Effect of varying quantities of borax on the growth of cotton on Colbert silt loam at Muscle Shoals, Ala., in 1920*

[Observations were made three weeks after planting.]

Quantity of borax applied per acre.	Fertilizers applied on May 10.		
	Sec. 1.—In the row and seed planted 10 days later.	Sec. 2.—In the row and seed planted immediately.	Sec. 3.—Broadcast and seed planted immediately.
No borax			
1 pound	Normal	Normal	Normal.
2 pounds	do	do	Do.
3 pounds	do	do	Do.
No borax			
4 pounds	Normal	Normal	Do.
5 pounds	do	do	Do.
10 pounds	do	do	Do.
No borax			
20 pounds	Slight retarding	Slight injury	Slight retarding.
30 pounds	Somewhat stunted	Plants small; many dying.	Do.
50 pounds	Germination low; plants show yellowing.	Germination low; plants dying.	Somewhat stunted.
No borax			
100 pounds	Germination about 50 per cent; plants dying.	Only an occasional seed germinated; plants, dying.	Germination decreased and plants dying.
200 pounds	Only an occasional seed germinated; plants about dead.	Seven seeds germinated; plants about dead.	Germination decreased about 70 per cent; most plants dead.
400 pounds	No germination	No germination	Twelve seeds germinated and plants died.
No borax			

The quantity of borax required to produce a noticeable injury to cotton receiving fertilizer in the row was 20 pounds. To lower germination appreciably and cause the death of any very large percentage of the plants 50 pounds were necessary. Where the fertilizer was used in the row and planting was delayed for 10 days the injuries seemed to be decreased about 50 to 75 per cent. Distributing the fertilizer broadcast, as in section 3, decreased the effects as much or possibly slightly more than delaying planting. It is shown that any method of use which decreased the concentration of the borax around the plant roots markedly decreases the injuries.

During the 10 days preceding the planting, May 1 to 10, 2.06 inches rain fell, and for the 10-day period following the planting 3.34 inches rain fell. On the second day after planting 1.6 inches precipitation occurred, which was followed by light showers for several days. On the seventh day after planting there was a rainfall of 1.56 inches. The total rainfall for the month was 5.70 inches.

Even with this heavy rainfall there was unquestionable injury from the borax with 30 pounds per acre. With 50 pounds per acre germination was low when the fertilizer was put in the drill and seed planted immediately, and many of the plants died after germinating. When the fertilizer was sown broadcast the plants were stunted. With 100 pounds and over per acre there was practically no germination. The section of the field where 100, 200, and 400 pounds of borax per acre were used is shown in Plate 2, B.

SUMMARY

Experiments were made to test the effect of borax in fertilizers on cotton at Arlington Farm, Va., and at Muscle Shoals, Ala. The soil at Arlington Farm is a productive silt loam suitable for truck and general farm crops. The soils on which the experiments were located at Muscle Shoals were the Clarkville loam and the Colbert silt loam. Borax was mixed with fertilizers and applied so as to add 5, 10, and 20 pounds of anhydrous borax per acre in two of the experiments. In a third test the quantities varied from 1 to 400 pounds per acre.

At Arlington Farm and Muscle Shoals borax in small quantities was injurious to the young plants.

On the silt loam at Arlington Farm, when the fertilizer was applied broadcast, 5 pounds of borax per acre were not always injurious, 10 pounds were slightly injurious, and 20 pounds were distinctly so. Application of 20 pounds reduced the weight of the green plants in the various experiments from 15 to 35 per cent. When the fertilizer was applied in the drill and seed planted immediately, 5 pounds was slightly harmful, 10 pounds was distinctly injurious, and 20 pounds caused severe injury, especially on germination and early growth. When the fertilizer was applied and allowed to stand until after a rain before the seed were planted, the effect was less severe.

On the Clarksville loam at Muscle Shoals a somewhat similar result was obtained. In one of the experiments, 5 pounds of borax applied in the drill with the fertilizer decreased the green weight of the plants 5.7 per cent, 10 pounds decreased the weight 12 per cent, and 20 pounds decreased it 39 per cent. The injuries to early growth were much more marked than these figures would indicate.

The influence of rainfall was noted. While the season was a normal one from June, the rainfall materially influenced the effect of the borax. Wherever a light rainfall occurred soon after planting and was followed by a dry period the effect was severest. If heavy showers followed periodically after planting the effect was less severe.

In an experiment started in the early spring on the Colbert loam at Muscle Shoals where the rainfall was very heavy for 10 days after planting, germination was materially affected where the fertilizers were applied broadcast by 100 pounds of borax per acre, and no germination took place with 200 pounds per acre. Where the fertilizers were put in the row, germination was materially affected by 50 pounds per acre and was prevented entirely by 100 pounds.

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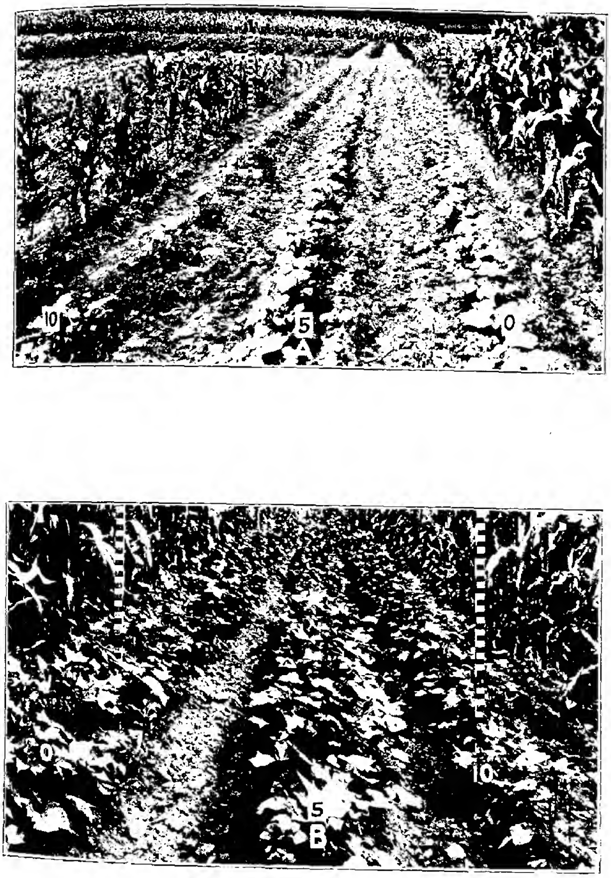
PLATE 1

Effect of borax on cotton in experiment A at the Arlington Experimental Farm.

A.—0, no borax; 5, 5 pounds per acre; 10, 10 pounds per acre. Photographed July 10, 38 days after planting.

B.—Experiment A, five weeks later. Photographed August 17, 75 days after planting.


10
5
A
0
0
10
5
B


0
5
10
20
A


B

PLATE 2

Effect of borax on growth and germination of cotton.

A.—Effect on cotton at the Arlington Experimental Farm in experiment C: 0, no borax; 5, 5 pounds per acre; 10, 10 pounds per acre; 20, 20 pounds per acre. Photographed 43 days after planting.

B.—Prevention of germination of cotton by 100, 200, and 400 pounds of borax per acre. The bare rows show where these large quantities of borax were used.

PLATE 3

Effect of borax on cotton in Section A in the Clarksville loam at Muscle Shoals, Ala. 0, no borax; 5, 5 pounds per acre; 10, 10 pounds per acre; 20, 20 pounds per acre.

A.—Photographed July 29, 47 days after planting.
B.—Photographed August 26, 74 days after planting.
C.—Photographed September 18, 96 days after planting.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5
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A
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5
10
20
A
B
20
A
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C
5
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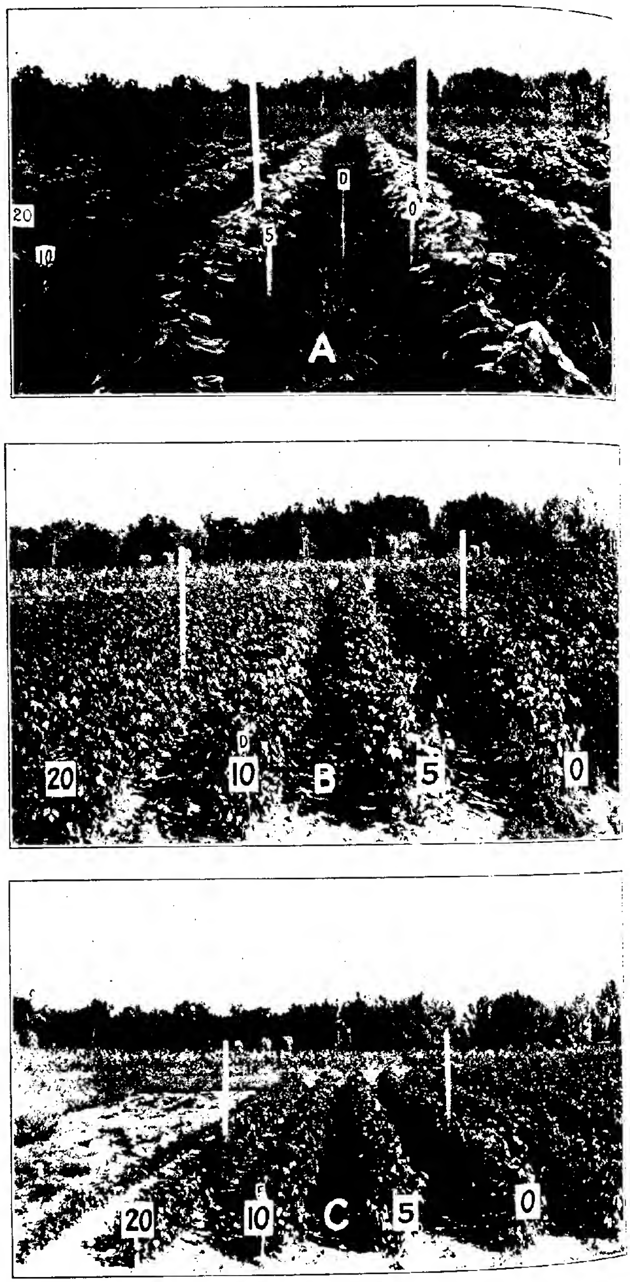
20
10
D
5
0
A
20
D
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B
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20
10
C
5
0

PLATE 4

Effect of borax on cotton on Clarksville loam in Sections D and F. 0, no borax; 5, 5 pounds per acre; 10, 10 pounds per acre; 20, 20 pounds per acre.

A.—Cotton in Section D. Photographed August 23, 50 days after planting.

B.—Cotton in Section D. Photographed September 18, 75 days after planting.

C.—Cotton in Section F. Photographed September 18, 58 days after planting.

GENETICS OF BUNT RESISTANCE IN WHEAT[1]

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INTRODUCTION

The losses due to bunt, *Tilletia tritici* (Bjerk.) Wint., in the Pacific Northwest have been steadily increasing for the past 25 years, notwithstanding the most earnest efforts on the part of scientists and farmers to reduce them. The seed has practically always been treated with blue vitriol or formaldehyde, but in spite of every precaution the winter wheat often contains from 10 to 50 per cent of bunted heads at harvest time, apparently due to soil infection from wind-borne spores. In the State of Washington alone the most conservative estimates place the losses at more than 1,000,000 bushels of wheat annually. The bunt problem has caused, and is causing, more interest and anxiety than any other of like nature through the winter wheat districts. Spring-sown wheat seldom produces a bunted crop if the seed has been carefully disinfected, the spores in the soil having perished during the winter.

The work has been closely linked with the other cereal and pathological investigations at the Washington Agricultural Experiment Station, and many friends and coworkers have contributed to the material in hand. Acknowledgment is here made to the various members of the staffs of the Washington Station and the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture, for their ever-ready help and enthusiasm; to E. M. East for helpful suggestions and criticism of the manuscript, and also to H. B. Humphrey, H. M. Woolman, E. G. Schafer, and F. D. Heald for suggestions and corrections in the presentation of the subject matter.

When the writer became assistant cerealist of the Washington Agricultural Station in 1911, many inquiries were coming in from farmers concerning control measures for bunt in winter wheat. The limitations of seed treatment were well known, and soil sterilization seemed impracticable under the extensive system of wheat raising on the great farms of the winter-wheat belt. The possibility of obtaining or developing a strain that would resist the attacks of the fungus and at the same time fulfill the requirements of winter hardiness, prolificacy, milling quality, etc., seemed the most promising solution of the problem. Accordingly hundreds of varieties from the principal wheat districts of the world have been introduced and many hybrids have been made in an attempt to find or develop such a wheat. Preliminary reports (*13, 14*)[2] of this work have already been published.

The following paper deals with that part of the investigation concerning the comparative resistance of different wheats and the inheritance of the factors that cause resistance, as expressed in the hybrid segregates of succeeding generations.

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[2] Reference is made by number (italic) to "Literature cited," p. 476-479.

RESISTANCE PHENOMENA IN GENERAL

RELATIONSHIPS AND RESPONSES OF HOST AND PARASITE

It has long been known that plants and animals vary greatly in their susceptibility to disease, and that the causal organisms are more or less restricted in their choice of host. This variation may be attributable to the inability of the causal organism to set up a pathogenic relationship with the host or to its inability to function after having established such relationship. Failure to infect may be due to an incompatible optimum of temperature, moisture, light, or nutrient materials. These factors may also provide the physiological basis for failure to function after infection has taken place. In addition, the outer coat may offer a mechanical obstruction. Thus, the bark of trees, the seed coats of grain, and the skins of animals offer a powerful protection against invading organisms which gain a ready access if injury occurs. However, a great majority of the pathogens that have been studied experimentally seem to have less difficulty in gaining entrance to closely related resistant forms than in continuing that existence after having gained entrance. Thus in Marryat's experiments (*32*) infection by yellow or stripe rust [*Puccinia glumarum* (Schm.) Erikss. and Henn.] occurred with equal readiness through the stomata of resistant and susceptible wheats. The susceptible Michigan Bronze wheat seemed to nurture the infection tubes and hyphae, the cells accepted the haustoria without shrinking, and normal spores were soon produced. Hyphae never seemed to flourish in the resistant Einkorn, but appeared stunted and watery and seldom produced haustoria. They soon died, but in dying they killed the host cells with which they came in contact—appearing to be mutually toxic. The American Club, intermediate in resistance, sometimes killed the fungus without reducing the vigor of the host cells, but in other places the hyphae were found flourishing while the host cells seemed to be dying. When spores did develop there was not enough force to break the epidermis. Apparently resistance in this case was produced by antitoxins furnished by the host and toxins furnished by the parasite. Ward (*60*), also working on the histology of resistance, showed that size of stomata, hairs, or comparative leaf surface had no influence whatever on the susceptibility of different bromes to brown rust, *Puccinia dispersa* Erikss. and Henn. After treating a mass of data statistically he drew the following conclusions:

> Resistance—is not to be referred to observable anatomical or structural peculiarities but to internal, i. e., intraprotoplasmic, properties beyond the reach of the microscope, and similar in their nature to those which bring about the essential differences between species and varieties themselves.

This was in 1902. Since then a great mass of experimental work in plant pathology, bacteriology, and medicine has added weight to his conclusions. For example, Stakman (*45*), who has done much work on the phenomena of resistance in cereals and grasses to the stemrust, *Puccinia graminis* Pers., concludes from his histological study of hyphal invasion (to quote his summary):

> 1. When plants practically immune to *Puccinia graminis* are inoculated, the fungus gains entrance in a perfectly normal manner.
> 2. After entrance the fungus rapidly kills a limited number of the plants cells.
> 3. The fungus, after having killed the host cells in its immediate vicinity, seems unable to develop further.
> 4. The relations between plant and parasite in partially resistant and almost wholly immune plants are different in degree only.

5. Hypersensitiveness of the host seems to be a common phenomenon not only among plants somewhat resistant to *P. graminis* but also among those almost totally immune to it.

These conclusions point to the probability that resistant and susceptible plants differ in the chemical composition of their cell walls or protoplasm or both. Valuable information might be gained along these lines by such investigations as the recent work of Rumbold (*43*) and Thiel and Weiss (*55*). Rumbold killed Chestnut trees by injecting an extract of the cankerous tissue caused by the chestnut-blight fungus [*Endothia parasitica* (Murr.) P. J. and H. W. Anderson] into the trunks of healthy trees. Extracts from healthy tissue produced no effect.

Thiel and Weiss were able to germinate teliospores of *Puccinia graminis tritici* Erikss. and Henn. in midwinter by soaking them 15 minutes in 1 per cent citric acid, although untreated spores would not germinate at all until spring. Other acids, lipoid solvents, and sodium hydroxid were ineffective. From this it would seem that the acid had acted as a specific activator through the cell wall on the protoplasm. Susceptible hosts may contain similar activators which stimulate the metabolism of the parasite.

The delicate chemotactic balance between host and parasite as an explanation of the differing degrees of resistance finds support in the investigations of Spinks (*44*) who found that nitrates, and especially lead and zinc nitrate, increased the susceptibility of wheat and barley to mildew and rust. Furthermore, potash and lithium salts decreased susceptibility; but resistant varieties maintained their relative resistance under either condition.

From this it is logical to assume that species or races which vary in their resistance to disease-causing organisms when growing under identical environmental conditions do so because of their physical and chemical individualism. If this be true, then the complex of causes responsible for resistance and immunity in plants is very similar to, if not identical with, those producing resistance and immunity in animals.

The classic researches the last 25 years in the antitoxin laboratories have shown that susceptibility to disease is caused by the physicochemical reactions of poisons, toxins, ptomains, etc., produced by the causal organism in the various tissues of the host. In acquired immunity the host cells have the power to develop counteracting chemicals which neutralize the harmful substances produced by the pathogene. In natural immunity either the counteracting chemicals are already present, the temperature, light, food, etc., are not optimum for the pathogene, or else the composition of the protoplasm of the host is such that no chemical reaction takes place. An illustration of the latter condition is described by Zinsser (*62*) regarding tetanus from the work of Blumenthal (*6, p. 185*). As is well known, the central nervous system is the special point of attack for tetanus poison, and Blumenthal found that tetanus toxin was neutralized by the brain tissue of susceptible animals, but the brain tissue of resistant animals like the chicken had little or no neutralizing power. The frog is also immune to tetanus under normal conditions. When the toxin is injected, the body temperature being too low to allow the combination between toxin and nervous tissue to take place, no tetanus results. If, however, the frog is placed in a tank of warm water and kept at the normal temperature of the human body it succumbs to the effects of the toxin. Clearly chickens and frogs are

resistant to tetanus for very different reasons. The first, because of the different chemical compositions of the brain; the second, because of physical incompatability to the parasite.

Zinsser in discussing nonheritable natural immunity, says:

> The individual differences in resistance which unquestionably exist among members of the same species and races are very difficult to explain, but, as far as we can tell anything about them at all, they seem to depend upon variation in what is popularly spoken of as "general condition."

This indicates that the counteracting chemicals are present in greater or less amounts even in organisms that readily succumb to a given disease. Or, stating it in another way, the particular tissue to be attacked can suffer injury and recover if the host is well nourished and in an optimum environment for its existence. This phase of natural immunity leads directly into the phenomenon of acquired immunity.

Injuries or wounds in plant and animal life not only stimulate repair but also growth and rejuvenescence. If the injury is sufficiently severe, or repeated often enough, the energy required to stimulate the affected part is too great a tax and the organism dies. Any factor which reduces the energy of the organism, such as fatigue, malnutrition, unfavorable temperature, etc., should reduce its resistance to disease, and this is what actually happens.

In naturally acquired immunity, the injury caused by the toxin of the invading organism stimulates an overproduction of the material used up in neutralizing it, so that subsequently more and more of the toxin can be neutralized without fatal results. Similarly in induced immunity, the injection of increasing amounts of toxin can be borne owing to the preponderantly greater amounts of neutralizing substances elaborated. In immunization work, one of the standard methods is to inject sublethal doses of fully virulent organisms in order to stimulate this great overproduction of these counteracting chemicals, which are cast into the blood and neutralize the counteracting poison at once. Such immunized animals may or may not, depending upon the particular antigen or toxin, retain this immunity for life.

Since the beginning of modern medical sceince it has been known that people having once recovered from such diseases as plague, cholera, smallpox, or yellow fever will not again during their lifetime contract it; but lasting immunity is not conferred by one attack of such diseases as pneumonia, tetanus, or influenza. In attempting to explain or make use of these phenomena a great branch of physiological chemistry has been developed.

The most comprehensive attempt to explain the causes of immunity is that offered by Ehrlich (*10*), in which he conceives the cells of host and parasite as aggregations of complex molecules which are themselves complex. Complex molecules react with one another through certain of their side chains, but only when these side chains have a certain definite correspondence in structure. The reactions of immunity represent only a repitition of the processes of normal metabolism. A receptor is an outlying part (the chain) of the cell which is able to combine, by means of a so-called haptophorous group, with foreign (protein) molecules. The haptophorous group of the receptor is able to combine with a food molecule or with a toxin molecule. Such combination stimulates the cell to produce more receptors which, in the case of toxin combination, may result in over production, the

superfluous receptors becoming detached from the cell. These are called amboceptors because they have two haptophorous groups, and in the immunizing process they act as links to bind the invading cells to the complements which are normally present and which, when so united to the foreign cells, are able to destroy them by means of a zymotoxic or toxophore group. The amboceptor with its complement constitutes a cytotoxin, hemolysin, or bacteriolysin. Other detached receptors act as antitoxins, as agglutinins, or as precipitins. Toxins have a haptophorous group by which they combine with antitoxins, and a toxophorous group to which their injurious effects are due. A toxin which has lost its toxophorous group, as by heating, is called a toxoid. A complement which has lost its zymotoxic group is a complementoid and an amboceptor that has lost one of its haptophorous groups is called an amboceptoid. The presence of a foreign cytotoxin leads to the presence of an anticytotoxin, which may act on the amboceptor or on the complement.

Whether Ehrlich's or similar theories can be extended to account for all the complex phenomena of reistance and immunity in plants and animals remains for the future to decide. Like the hypotheses in physics and chemistry, it offers a shorthand method for explaning the facts. It helps to make a clearer understanding of the phenomena of both natural and acquired resistance and immunity and also explains in a logical way the change in the virulency of organisms, in so far as there is any explanation at present. For example, as Walker (*59, p. 34*) has shown the pathogenic organisms themselves may be immunized against immune serums by cultivating them in media containing increasing portions of the immune serums.

It is not too much to suppose, therefore, that pathogenic organisms themselves are subject to contagious diseases, suffer from malnutrition, and benefit by becoming acclimated, in response to the same laws that operate in higher organisms. Changes in virulency can probably be explained as the interaction of these factors in the majority of cases; but the plasticity or heterozygous character of some forms offer the possibility of genetical change by selection. The brief time required for each generation in many of the lower forms and the large numbers produced would make this a quick and permanent method of adapting an organism to its environment. Since, in general, the lower forms are more constant or stable than the higher forms, it is probable that such genetic change by selection is very limited.

From the foregoing discussion it seems that disease-resistance phenomena in both plants and animals are due to the same general causes. The hosts of a given parasite may vary both morphologically and physiologically; and according to their physico-chemical complex react diversely in their irritability and response to such factors as temperature, moisture, light, nutrition, and poison. The same organism will react differently to the same stimuli at different stages in its development. In so far as the subject has been studied, the parasite or disease-causing organism seems to vary also, and according to the same laws that govern the host. Added to this, the host is changed within certain limits, chemically, by its food supply and other external stimuli, such as other diseases or mechanical injury. The parasite probably has its diseases and is no doubt changed chemically by them and also by its changing food supply. In the delicate balance between these two organisms, host and

parasite, there is a physico-chemcial complex so intricate that it has thus far been impossible to comprehend or to analyze it in all its detail. Heritable resistance represents a permanent upsetting of this balance in favor of the host, caused by the variation in its physico-chemical complex.

INHERITANCE OF RESISTANCE

Acquired resistance and induced immunity, like other acquired characters, have not been shown experimentally to be inherited in the ordinary sense of the term, but each generation must recover from a given disease or be vaccinated (or inoculated with the proper virus) in order to acquire immunity. Inherent or natural resistance is definitely passed on from generation to generation like morphological characters. When such a resistant race is crossed on a susceptible one, resistant and susceptible races may be isolated in subsequent generations, which show that the same laws of inheritance which control other characteristics are in operation in determining the causes of resistance. Biffn (*2*), in England, found resistance to yellow rust *Puccinia glumarum* Erikss. and Henn. and resistance to mildew *Erysiphe graminis* DC. in barley to be a simple mendelian recessive. One of his resistant hybrid wheats has become of commercial importance and has retained its resistance at Cambridge for 16 generations. Nilsson-Ehle (*34*), in Sweden, corroborated Biffen's findings as to the heritability of resistance to yellow rust, but in his crosses he obtained segregates more resistant and more susceptible than the parents, which he interpreted as being due to modifying multiple factors. Hayes, Parker, and Kurtzweil (*15*), in America, found the same phenomenon in connection with resistance to stemrust *Puccinia graminis* Pers. in wheat and in addition found linkage between resistance and certain durum and emmer-like characters.

Other genera that have been studied genetically in regard to resistance to various diseases, in general show the same type of inheritance. McRostie (*30*) ,working with two independent strains of the bean anthracnose organism *Colletotrichum lindemuthianum* (Sacc. and Magn.) Bri. and Cav., showed resistance to anthracnose to be dominant to susceptibility. When a bean resistant to both strains was crossed on one susceptible to both, a ratio of 9 resistant to 7 susceptible was obtained in the F_2 generation. Either strain alone gave the expected 3 to 1 ratio. Later (*31*) he gave additional data including 36 F_3 families which supported his earlier interpretation. In addition he reported rootrot (caused by *Fusarium martii phaseoli* Burk.,) and mosaic resistance in beans to be partially recessive. He interpreted his F_2 results according to the ratio 9 susceptible to 7 resistant, which was supported by the performance of 183 F_3 families for rootrot and 329 F_3 families for mosaic. Parker (*39*) published a preliminary report on the crownrust of oats, *Puccinia lolii avenae* McAlpine, in which he concludes that resistance is recessive but was caused by multiple factors. Orton (*36, 37*) described the transference of wilt resistance of the stock melon to the watermelon by crossing, and similarly, resistance to wilt and rootrot was transferred from the Iron cowpea to one of the Whippoorwill type.

Among the farm crop plants that have attained commercial importance through their disease-resisting qualities, but which originated by selection or introduction, may be mentioned the following:

1. Upland and Sea Island cotton resistant to wilt. (Webber.)
2. Potatoes resistant to late blight and scab. (Jones and Stuart.)

3. Flax resistant to wilt. (Bolley.)
4. Cantaloup resistant to leafblight. (Blinn.)
5. Clover resistant to anthracnose. (Bain and Essary.)
6. Durum wheat in the Dakotas (Carleton) and Kanred wheat in Kansas (Roberts) resistant to rust.
7. Tobacco resistant to rootrot. (Johnson and Milton.)
8. Grape resistant to Phylloxera. (Viola and Ravaz.)

A genetic analysis of the resistance of these crops after crossing them with susceptible races would be required to show the type of inheritance in each case. Such investigation would be more concise and the interpretation clearer if a standard method of inoculation were adopted and a quantitative rather than a qualitative measure of resistance were used, as is being done in rust resistant work with cereals (*38*). The analysis of the inheritance of rootrot in tobacco (*21*) should prove especially interesting because of the ease with which crosses are made and also on account of the amount of genetic work already done on tobacco. It is difficult to interpret much of the past work because inoculation was left to chance or natural agencies, which allowed part of the individuals to escape infection and the resulting segregates were arbitrarily classified as resistant or susceptible, when as a matter of fact every graduation from complete susceptibility to complete immunity occurred. In some cases (*35*) different stages in the life history of the organism apparently do not have the same infective power.

Whether resistance of a given host against a given parasite will be maintained indefinitely in a given environment remains for the future to decide. Resistance in certain crops (*46*) may be only temporary. The resistance of the host might be apparently lost through the introduction of a new physiological race of the parasite to which it was susceptible. An example of such an occurrence is recorded by Levine and Stakman (*25*). They obtained a strain of stemrust from Oklahoma that could attack Kanred wheat although Kanred continued to be very resistant to the two common races of stemrust in Kansas. According to Evans (*11*) Bobs Rust Proof wheat was resistant to stemrust at Pretoria, South Africa, but was badly attacked in the Low Country of the Transvaal. This may have been due to the change in environment or to the presence of a different biologic form of the fungus encountered in the Low Country. Evans also reported an extraordinary case in which the pathogenic properties of stemrust on wheat were increased nearly five fold by passing one generation of its existence on the F_1 generation of a cross between a resistant and a susceptible variety (Bobs × Wol Koran). Bobs normally produced one-fifth as many pustules as Wol Koran, but when infected with spores from the F_1 generation it produced one-third as many. In this case the F_1 generation had apparently acted as a "bridge" to permanently increase the virulency of the rust and had also decreased the difference between the resistant and susceptible varieties. If this should prove to be a general rule, breeding for resistance would not only be without avail but would actually be a very dangerous practice on account of the danger of developing and spreading super-virulent cultures of the parasite. It is to be regretted that Evans did not grow an F_2 generation of the host to see whether super-resistant segregates could be obtained. He worked with comparatively few plants in the greenhouse, and his results may have been due to some uncontrolled factor, for other

investigators have not been able to duplicate his results. Biffen (*3*) is positive that such "bridging" or increase in virulence did not occur among any of his many hybrids in England. Stakman and his associates (*45-53*) in America, after long and careful work with stemrust on its many hosts, have failed to find any indication of "bridging" or increased virulence of the parasite. They grew it on hybrids of resistant × susceptible wheat varieties from the F_1, F_2, and F_9 generations. They used different physiological races of the parasite and many species, including several genera of host material. In no case did they find an intermediary host that would increase the virulency of the rust. On the other hand, they found that the parasite became sickly when cultivated on a resistant host and often could not be maintained more than a few generations.

According to Vavilov (*58*) "bridging species" are found in *Puccinia Symphyti Bromorum* F. Müll. (M. Ward and Freeman), *Erysiphe graminis* DC. living on Bromus (E. Salmon), in *P. graminis forma* sp. *tritici* (Freeman and Johnson), and *Sphaerotheca Humuli* on Alchemilla (Steiner). It will be noted that these few cases deal with relatively weakley specialized fungi, when compared with the smuts, among which biologic races of different infecting power are known to exist; and the hosts used contain races varying from complete susceptibility to immunity. The conflicting accounts of American investigators may be explained on similar grounds. For example, Johnson (*20*) was able to transfer timothy rust *Puccinia phleipratensis* Erikss. and Henn. to barley by means of oats as a bridging host when it could not be transferred from timothy direct, but Stakman and Jensen (*47*) transferred timothy rust successfully to oats, barley, rye, wild oats, oat grass, orchard grass, wild rye, rye grass, and rough brome, without the use of a bridging host.

The biologic races of hosts and parasites, differing in resistance and virulency and the difficulty of keeping pedigreed cultures of each not only make accurate work difficult but make it almost impossible for two investigators working in different parts of the country to corroborate each other's work. "Wheat" and "stemrust" are terms not sufficiently specific for work of this kind. The particular variety of wheat and the biologic race of the rust must be known.

Plants vary also in their resistance to the attacks of insects. Torssell (*56*), in Sweden, found certain winter wheats that were not harmed by the six-spotted leafhopper while other varieties in the same field were destroyed. In 1916 in the variety test of field peas planted at the Washington Agricultural Experiment Station, the seed of Alaska peas was infested with pea weevil. At harvest time the Alaskas contained more infested than good seed, while the Bangalias growing beside them contained only a trace. The adult weevil is winged but evidently preferred the Alaskas. It is a common observation among potato growers that the Colorado potato beetle will destroy certain varieties before attacking others. For example, if Early Rose and Rural New Yorkers are planted in alternate rows in a garden, the beetles will strip the leaves of the Early Rose before harming to any appreciable extent the Rurals. But perhaps the most striking example of resistance to insect attack is that of certain grapes resistant to the plant louse *Phylloxera vitifoliae* Fitch. described by Bioletti (*4*). When resistant and susceptible varieties are crossed, resistance seems to be dominant. Such hybrids have become the foundation stock for grafting with the choice wine, table, and raisin grapes of Europe. Phylloxera feeds on the

roots of the grape and produces galls or "tuberosites" which, on the susceptible varieties, enlarge enormously, and in two or three years kill the vines. This growth is caused by a poison which is injected by the insect. The resistant varieties do not react to this poison, and in addition the insect does not multiply so rapidly on it, which indicates a repellant effect of the juice of the resistant plant on the life of the Phylloxera. Between two and three million acres of vineyards in France and California have been destroyed by Phylloxera during the past 50 years and have been replanted to choice varieties that have been grafted on to resistant stocks.

Little work has been done on the inheritance of disease resistance in animals. The nearest approach to an exhaustive critical investigation in this subject, is that by Little and Tyzzer (*26*). They could transplant the carcinoma tumor designated as *J. w. A.* at will on the Japanese waltzing mouse, but the common mouse was immune to it. The F_1 generation of these two races was very susceptible, there being but 1 in 62 that failed to react. The F_2 and F_3 generations were very nearly immune, there being but 3 reacting positively out of 221. The F_1 generation, back-crossed with the susceptible parent gave mostly susceptible offspring (64 positive to 4 negative), but when back-crossed with the immune parent only immune offspring were produced (112 negative reactions). Altogether 629 mice were used in the investigation. The authors interpreted susceptibility to transplanted carcinoma tumor *J. w. A.* as being due to a complex of dominant multiple factors but considered their data insufficient to arrive at an approximation of the number involved.

From the illustrations given above it will be seen that the researches already made on the inheritance of disease resistance indicate that, in the main, whether due to physical or chemical causes, Mendelian laws of segregation and recombination control resistance phenomena generally. Artificial crossing and selection of large numbers should help clear up the difficulties in making practical use of these laws in controlling crop and animal pests.

BUNT RESISTANCE PHENOMENA IN WHEAT

THE PARASITE

There are two species of bunt, *Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kühn. *T. tritici* is the most common and is the one generally referred to in the literature, and, unless otherwise designated, is the species referred to throughout this paper.

The smuts (order Ustilaginales) were among the first recognized plagues of plants. Pliny (*41*) and Theophrastus (*54*) wrote about smut outbreaks in grains and considered them due to untoward weather and soil conditions which caused a morbid transformation, that is, putrifaction, of the tissues of the grain. In 1801 Persoon (*40*, p. 224) described stinking-smut as being due to a fungus. Meyen (*33*, p. 108–119) as late as 1841 wrote that the smut powder in a dissolved condition entered the roots and caused the sap to engender the same disease. But Kühn (*23*) was the first to prove that the fungus from the germinating spore actually enters the young wheat plant, thereby starting infection that results in the smutted head.

According to Arthur (*1*) farmers reported a bunt outbreak in 1859 in the Haw Patch district of Indiana that took half the crop. Although such epidemics are not common in Indiana, another one occurred in this same district in 1887 and in some fields caused an almost total loss.

In total harm done in reducing the world's wheat crop, bunt is second only to rust (*19*). In the wheat section of the Pacific coast in America in 1919 it is estimated that between two and three million bushels were lost on account of it. Besides the actual reduction in yield, the spores clinging to the grain and straw may be harmful to certain animals, and added expense is required to clean the grain for flour if the crop is even slightly contaminated. It is known and dreaded in all countries where wheat is grown. The countless spores in the separator during thrashing are sometimes exploded by the static electricity of the revolving cylinder and a fire is started that destroys both machinery and crop (Pl. 1, A). During thrashing the liberated spores are disseminated by the wind, and, as Heald and George (*16*) have shown, distributed at the rate of more than 35,000 spores to the square inch during a single season over distant fields about to be planted to fall wheat. Under such conditions seed treatment will not prevent a smutty crop of winter wheat the following year if the season is favorable for infection by the wind-borne spores. Experiments have shown that in a few weeks after the first rains, however, the spores germinate, and in the absence of wheat seedlings to infect, the fungus dies within a month or 6 weeks. Spores have been kept in the dry for 12 years which would still germinate (Woolman and Humphrey.[3])

McAlpine (*28*, *29*) describes the spores of *Tilletia tritici* (Bjerk.) Wint. as globose with reticulate roughened surface 15 to 22 μ (average 16μ) in diameter. Under suitable temperature and moisture conditions the resting spores germinate in air or water. In water a long septate, unbranched mycelium is produced, whereas in air a short, septate, promycelium is produced, at the end of which are borne 3 to 10 filamentous or sickle-shaped conidia. These conidia, fusing in pairs, produce the infection threads by means of which the parasite enters the host. This is accomplished between germination and the beginning of photosynthesis of the young wheat plant. Once established, the fungus grows until fruiting time, keeping pace with the growing point without serious injury to the host. It then produces a mass of hyphae in the walls of the young ovules, which results in such a quantity of odoriferous spores as to fill the ovaries to the point of bursting—that is, making "smut balls" about the size of wheat kernels.

The resting spores are never produced except in the ovaries of the host. The parasite remains in a vegetative condition during the lifetime of the host—whether it be 2 months or 10. The physico-chemical changes that take place in the wheat plant as it approaches maturity stimulate the bunt organism to similar changes, for it quickly spreads its hyphae through the remains of the nucellus and deposits masses of its own fruiting spores in the space normally occupied by the endosperm and embryo of the wheat.

Bunt, in common with other smuts, apparently consists of but a single biologic race. Like other disease-producing organisms, it is selective for certain tissues (*24*). The nodes and growing points of the host are the

[3] WOOLMAN, Horace M., and HUMPHREY, Harry B. STUDIES IN THE PHYSIOLOGY AND CONTROL OF BUNT OR STINKING SMUT OF WHEAT. U. S. Dept. Agr. Bul. (In press.)

tissues in which it is most frequently found in this case, and the fungus no doubt produces toxins which bring about the deformity characteristic of a smutted plant. (Pl. 1, B; 2, A.)

According to Woolman,[4] bunt infection takes place during the early seedling stages, through the walls of the coleoptile. He is unable to find any trace of mycelium except in the coleoptile until the seedlings are about 8 days old. By the time they are 15 days old the mycelium has penetrated to the inner side of the second leaf sheath. As the plant grows older the mycelium penetrates farther and is carried upward mechanically with the growing point, living filaments being found most frequently at the nodes and in or near the actively growing parts. The older parts of the mycelium disappear, apparently being digested and absorbed. He finds that the so-called immune varieties are readily infected and that resistant and susceptible seedlings show no difference during the first two weeks. By the time they are a month old, however, the mycelium in the resistant varieties is more restricted in its ramifications and appears lacking in stainable cell contents. The "resistance" is evidently chemical in nature and begins to restrict the growth of the mycelium shortly after the genesis of photosynthesis. He rarely finds evidence of infection at more than one point, although he found one head containing spores of both *Tillitia tritici* and *T. levis*, which proves that two infections did take place, each functioning at maturity.

Heald (*17*) believes that multiple infection is required to develop a smutted plant. He found that Marquis wheat, which is very resistant to bunt, could endure from 500 to 1,000 spores per grain without producing bunted heads in the succeeding crop. When seed carrying 100,000 or more spores per grain were planted half the resulting crop consisted of bunted heads. Jenkins Club, which is very susceptible, produced a trace of bunt with as few as 100 spores per seed. When the inoculation reached 40,000 or more the resulting crop produced from 80 to 100 per cent of bunted heads.

If the multiple infection hypothesis be the correct explanation of this phenomenon, it might be compared to induced immunity in animals brought about by sublethal injections of fully virulent organisms. Infection in a great number of different places—that is, a lethal dose—would use up the "antitoxin" already present in the plant so rapidly that the pathogenic relationship could be established. The resistant varieties would either contain more of the "antitoxin" or a greater capacity for developing such material. Much more work will be required to establish this point. The data as published (*17*) might be interpreted as increasing the chances for infection or producing the external conditions that would make possible the penetration of the host cuticle (*7*). It has been shown that the amount of bunt is greatest in wheat planted for September 15 and only slightly less on October 1, at Pullman, Wash., the amount growing less and less in plantings either earlier or later. In view of this fact, as well as the fact that varieties differ in relative susceptibility, makes the proposition of determining by a spore count whether a given lot of seed needs treating, a difficult and precarious one, for winter wheat. A general prediction of the amount of smut to be expected in spring wheat would be less hazardous.

[4] My former colleague, Mr. H. M. Woolman, has done much cytological work on the relationships of bunt and wheat, and he has kindly permitted me to make the above statements of his unpublished work.

Summarizing the evidence at hand, bunt appears to be a highly specialized parasite, consisting of but a single biologic race and existing on but a single host genus, Triticum.[5] Its spores are disseminated both on the seed of the host and by wind to fields of fall wheat. Infection takes place only during the seedling stage of the host and to be effective may require multiple infection. It produces but one crop of spores a year, these being deposited in the wheat ovaries following the flowering period. Next to rust, it is the most destructive parasite of wheat. Besides the actual loss due to bunted heads, additional losses occur through feeding smutty grain to animals, through lowering the vitality of the seed by treatment with disinfectants, through the time and expense of treating the seed, through fires caused by explosions of spores during thrashing, through extra cleaning required in milling, and through experiments of farmers, such as planting out of season, changing seed, and trying other unadapted plants in an effort to avoid a smutty crop.

THE HOST

There are eight commonly recognized species of wheat in American agricultural literature. According to Tschermak (57) Einkorn, *Triticum monococcum* is quite distinct from the others and generally will not cross with them, although Blaringhem (5) succeeded in crossing it with durum and Polish wheat. Emmer, *T. dicoccum* Schr., is thought to be the progenitor of the other six. Durum, *T. durum* Desf., Polish, *T. polonicum* L., and Poulard, *T. turgidum* L. are inter-fertile but show about 50 per cent sterility when crossed with the other three. Common wheat, *T. vulgare* Vill., Club wheat, *T. compactum* Host., and Spelt, *T. spelta* L. are all fertile inter se.

Triticum generally shows intersterility with other genera, although crosses have been made with Secale (rye), but the F_1 generation is nearly always sterile, rarely producing seed. Love and Craig (27) reviewed former work on wheat-rye hydrids and reported a cross between Dawson Golden Chaff and common rye in which they obtained a single plant in the F_1, F_2, and F_3 generation, there being but one seed produced in each of the first two generations. The F_3 generation was not so nearly sterile, for it produced a number of seeds from which a variable population of F_4 segregates were obtained.

In common with many other members of the grass family the bread wheats have hollow stems closed at the nodes and two-ranked parallel-veined leaves consisting of sheath and blade. The sheath envelopes the stem with the edges overlapping. The flowers are perfect, arranged 1 to 6 on a spikelet, and the spikelets alternate on the rachis to form a spike. Two empty glumes inclose each spikelet. Each flower is inclosed in a floral glume on the outside and a palea on the inside and consists of three stamens with slender filaments and a 1-celled ovary beneath two styles with plumose stigmas. Wheat generally is self-fertilized, but natural hybrids sometimes occur in arid climates.

After germination the plant passes through a vegetative stage in which it produces a cluster of leaves (the stool), tillering from a zone of active

[5] Since completing this manuscript I found two smutted ryelike plants in an F_2 generation of Hybrid 128 wheat Rosen rye. The characteristic odor and reticulated surfaces of the spores make it almost certain that the casual organism was *Telletia tritici*, (Bjerk.), Wint. The same season (1921) F. J. Stevenson found two heads of what looked like bunt on common rye in our cereal nursery at Pullman, Wash., near the place where the two rye-wheat hybrid plants had been found.

tissue near the surface of the earth. With the approach of summer the stems elongate rapidly, the seeds develop, and the plant dies. The vegetative stage is completed in from one to eight months, depending upon temperature and variety.

The vegetative period exhibits some interesting variations. A fundamental varietal agronomic difference of great practical importance, which is entirely ignored in most taxonomic nomenclature, is the habit of growth during this vegetative period that distinguishes winter and spring wheats. A typical winter wheat, in the north temperate zone, will not head out or produce grain if planted in the spring. It remains as a green grass clump all summer and dies the following winter. If it is pastured or the leaves frequently cut off during the summer, it may live through the winter and the following summer head out and produce a normal crop of grain. Spring wheat, planted in the spring, passes through the vegetative stage in a few weeks and then the culms rapidly elognate, the head develops, and a normal crop is produced. If both are planted in the fall, the winter wheat lives through the freezing and thawing of a severe winter and produces a normal crop the following harvest under conditions in which the spring variety would winterkill. The time required from flowering to the maturing of the grain varies greatly with the changing climatic conditions. High temperatures, bright sunlight, and strong winds favor rapid maturation. Cool, cloudy weather with little wind favors the maximum length of time between flowering and maturity. The elongation of the stems, flowering, and fruiting are completed in from 30 to 60 days or longer, depending upon weather conditions.

Most of the cultivated wheats are of the species *Triticum vulgare*, but in certain districts the durum and club wheats predominate. Durum is produced in the Dakotas and western Minnesota principally, and the club area is confined, for the most part, to the Pacific Northwest.

Many investigators have classified comparative susceptibility to disease according to genera and species. (Jaczewski (*18*), Carleton (*8*), Freeman and Johnson (*12*), Stakman (*52*), Kirchner (*22*), and Reed (*42*).) For wheat they have shown that einkorn is generally resistant to all rusts and mildews. The durums, polish, poulard, and some of the emmers are partially resistant, but the common and club wheats, the spelts and part of the emmers are generally classified as susceptible. This shows a remarkable parallelism with the sterility groups and suggests the probability that the physical and chemical factor differences that cause sterility are also responsible for the differences in resistance. It would be expected, if this were the case, that specific sterility factors would be linked with resistance, and both would be linked with the morphological differences that make up the species. Hayes, Parker, and Kurtzweil (*15*) actually found such linkage, together with the expected sterility, in crosses of common with both durum and emmer. Linkage was not complete, however, for out of a large number they obtained a very few segregates of common type that were resistant to stemrust and some of emmer and durum type that were susceptible. From this it appears that the physico-chemical factor complex responsible for sterility in crosses of so-called immune and susceptible races is not identical with those producing resistance, although very closely associated. In fact, certain sterility factors may be the identical factors that cause certain kinds of resistance, for resistance is specific for each parasite. A wheat may be susceptible to stemrust and resistant to yellow rust, or resistant to mildew and susceptible to both rusts (*2*, *9*).

Bunt, like the rusts, finds certain species more congenial hosts than others. Table I, compiled from the tests of 1919 and 1920 at the Washington Agricultural Experiment Station, Pullman, Wash., shows the comparative resistance of the eight species as tested.

TABLE I.—*Comparative bunt resistance of the eight species of wheat*

Species.	Number of tests.[a]	Total number of plants counted.	Percentage of bunt produced.
Einkorn (*T. monococcum*)	4	87	0.0
Polish (*T. polonicum*)	3	66	7.7
Emmer (*T. dicoccum*)	10	155	9.6
Spelt (*T. spelta*)	21	515	9.7
Durum (*T. durum*)	40	1,256	29.3
Poulard (*T. turgidum*)	9	264	33.5
Club (*T. compactum*)	98	4,296	64.1
Common (*T. vulgare*)	666	25,009	70.2

[a] The actual number of distinct races or sorts was but slightly over 500 in round numbers. Many of the 1919 plantings were duplicated in 1920.

Taking the average percentage of bunt produced as the index of resistance, the eight species may be arranged into four groups. Einkorn is immune, polish, emmer, and spelt are very resistant, durum and poulard are intermediate, and club and common are very susceptible. It would require a much more exhaustive test to establish these findings as general laws for specific bunt resistance, but there are two deductions that may be made. 1. Species of Triticum differ in their susceptibility to bunt and in a manner analogous to specific rust resistance and also analogous to their genetic relationships. 2. Spelt forms a notable exception, falling into the very resistant class instead of the susceptible class that its genetic and rust-resistance relationships call for. These relationships are established by the work of several investigators (*15*, *57*, *58*), and the different behavior of spelt in respect to bunt resistance is established in Table 1 by the performance of 21 rows and more than 500 plants.

It has been found that the common and club wheats vary within rather wide limits among the different races of the same species, a few selections of both being very resistant, more being intermediate, but the majority being very susceptible.

Resistance to some diseases seems to break down when the host is grown in another environment. The information available on bunt resistance indicates that a wheat resistant to bunt will remain so in all climates. For example, Turkey (Washington, No. 326) is resistant under conditions favoring maximum infection at the experiment stations of Kansas, Minnesota, California, Oregon, Idaho, and Washington.

A comparison of a large number of varieties, known to be pure lines, in two districts of distinctly different climate should give a clear idea of the probable constancy of bunt resistance in different environments. This can best be done by means of a correlation table in which the comparative resistance of each variety in one locality is measured in relation to its comparative resistance in the other locality. If there is perfect agreement, the coefficient of correlation is 1. If they vary independently in each locality, without regard to the comparative resistance

in the other, the coefficient of correlation equals 0. Table II presents such a correlation.

TABLE II.—*Correlation of 150 varieties of wheat in respect to bunt resistance* [1]

Class centers.	5	15	25	35	45	55	65	75	85	95	Pullman frequencies.
5	4	....	1	....	1	....	....	....	....	....	6
15	1	1	....	1	....	....	....	....	....	....	3
25	1	....	2	....	....	....	1	....	2	....	6
35	....	1	....	....	2	....	....	3	....	....	6
45	....	....	2	3	....	1	2	1	1	....	10
55	....	1	....	....	1	2	1	1	....	2	8
65	....	....	1	2	2	5	2	2	4	4	22
75	....	1	....	2	1	2	....	4	5	1	16
85	1	....	1	....	1	1	3	4	6	5	22
95	....	....	....	1	1	....	1	6	12	30	51
Moro frequencies.	7	4	7	9	9	11	10	21	30	42	150

[1] Percentage of bunt at Moro, subject; percentage of bunt at Pullman, relative; 1919.
Coefficient of correlation = 0.654 ± 0.0315.

A comparison of 150 selections of wheat (mostly *Triticum vulgare*, a few being club and durum) tested at Pullman, Wash., and Moro, Oreg., show a correlation coefficient, according to Table II, of 0.654 ± 0.0315. Moro has an arid climate with an average rainfall of 11.6 inches. The elevation is 1,800 feet; the soil, a fine silt loam. Pullman has an annual rainfall of 21 inches, a clay loam soil, an elevation of 2,500 feet, and a lower summer temperature than Moro. The Moro data were obtained by counting the heads of bunt and wheat from which the percentages were figured. The Pullman figures were obtained by the combination plant and head count described on page 460. Taking into account the differences in climate and methods, the high correlation is significant. It shows that resistance and susceptibility are fundamental differences and are not easily changed by environmental conditions. A large number of these varieties have been tested at Davis, Calif., Aberdeen, Idaho, and Corvallis, Oreg., under wide differences of soil and climate, and the indications are that the common varieties that are decidedly resistant or susceptible are outstanding in those respects at all places where tested. Table II shows only four varieties less than 10 per cent bunt at both Stations, but 30 varieties more than 90 per cent susceptible. The average of all varieties at both Stations is 70 per cent, which is very near the figure given for the *T. vulgare* group in Table I, in which a much larger number of varieties is represented, covering the two-year test (1919 and 1920) at Pullman.

It is very seldom that a row of wheat, containing, say, from 50 to 100 plants produces nothing but bunt heads, no matter how susceptible the variety is or how favorable the conditions for infection are. A small number of wheat heads, and usually a few plants, escape the fungus and produce normal seed. It might be assumed that these occasional plants were resistant mutants. To test this possibility 10 bunt-free plants of hybrid 143 were selected in 1913, and for three years smut-free plants from the most resistant row were selected. At the end of the third year the selected plants produced 85 per cent of bunt as compared with 80

per cent for the unselected check. Other tests of selections from Turkey and Red Russian add proof to the conclusion that the bunt-free plants in varieties planted under conditions favoring maximum infection are not resistant mutants but rather escape infection by accident.

BREEDING FOR BUNT RESISTANCE

METHODS

In testing different wheats for comparative resistance to bunt, conditions favoring maximum infection were, as nearly as possible, maintained. The seed was inoculated with fresh viable spores just before planting. This was accomplished by stirring into each packet of seeds from 1 to 5 per cent of its weight in bunt spores, so that each seed was literally blackened by spores clinging to it, besides a surplus of loose spores among the seeds. The inoculum was obtained from the smutted heads of many different varieties of wheat in the field, in order to get a representative sample of the organism in its native environment. The heads were ground up and the spores sifted out and kept in a cool, dry place during the planting season. The packets of inoculated seeds were planted by hand in rows 18 inches apart. The seeds were spaced 4 to 6 inches apart in the row to avoid confusion in separating the plants at harvest time. Each experiment, whether a variety test or an F_3 family, was planted at approximately the same date to avoid error due to changing seasonal conditions. As many as 10 men were required at times in order to plant a given experiment in a single day.

The field was kept free of weeds and volunteer grain by rotation and cultivation, corn and field peas preceding the cereal nursery in a 3-year rotation in every case. The rainless summers of eastern Washington make clean cultivation under these conditions comparatively easy. A stake bearing a printed label with date, name of experiment, name of variety and pedigree number was placed at the end of each row. The records at harvest time were obtained as follows: To get a quantitative measure of resistance, the plants of each row were pulled and separated into three piles (bunt-free, all bunt, and part bunt); and the number of plants in each was recorded in a field note book. The partly bunted plants were then divided into heads of wheat and heads of bunt, and the numbers were entered in their proper columns. With these five figures it is easy to reduce the amount of bunt in a given row to terms of a single number for direct comparison with the amount produced by any other row. The computation used gives the total bunt in terms of percentage of the whole row, according to the formula $ab+c=d$, in which a is the percentage of bunted heads on the partly bunted plants, b is the percentage of partly bunted plants in the row, c is the percentage of entirely bunted plants in the row, and d is the total percentage of the row that is bunted. The computations were made with a calculating machine and checked with a slide rule. The results thus obtained are not materially different from those obtainable by a straight head count of all the heads in the row. This method takes much less time and gives information that a straight head count would not give—that is, the quantity of partly bunted plants and the amount of wheat produced on them, which in itself is an important measure of resistance, and is useful in checking errors in counting or computations.

The work was all carried on out of doors, in the field, the main care being to have uniform conditions for all the rows of a given test, so that

the differences in bunt resistance would be attributable directly to differences in the constitutional make-up of the wheats compared.

PARENT STOCK

Of the eight varieties to be discussed in relation to their bunt resistance, six are of wide commercial importance. Hybrid 128, Turkey, Fortyfold, Red Russian, and Jones Winter Fife are the five most important winter wheats in the State of Washington and are named in the order of their importance from the standpoint of total production. They represent more than three-fourths of the winter wheat produced in the Northwest. Alaska is not commercially grown except in isolated sections where an occasional farmer has been the victim of propagandists who have made extravagant claims for it as a wonderfully productive and hardy wheat. Of the two spring varieties, Marquis is third in production in Washington and is universally grown in the Great Plains area of the United States and in northwestern Canada. Florence is of some importance in Australia but has not been grown in America except for experimental purposes.

Taxonomically all are *Triticum vulgare* except Hybrid 128, which belongs to *T. compactum* and Alaska which is a poulard, *T. turgidum*. Turkey, Florence, and Alaska are resistant (Pl. 2, B) and Hybrid 128 and Jones Winter Fife are susceptible to bunt under all conditions. Fortyfold and Red Russian are intermediate but approaching the susceptible varieties (Pl. 3, A). Marquis is resistant when sown in the spring but intermediate or susceptible when fall-sown. Table III gives the amount of bunt produced in the tests from 1915 to 1920. The season of 1917 was very unfavorable, and the wheat did not come up till spring. Since none of the varieties produced any appreciable amount of bunt, the record of that year is omitted.

TABLE III.—*Annual percentage of bunt produced on parent stock for the years 1915 to 1920, with the 3-year average for 1918 to 1920, and the total number of plants in the tests during the latter period*

Variety.	Percentage of bunt.						Total number of plants in 3 years 1918–1920.
	1915	1916	1918	1919	1920	Average 1918–1920.	
Turkey	4.6	1.7	0.6	2.4	7.6	3.5	2,883
Marquis [a]	....	....	3.4	7.3	3.5	4.7	813
Florence	....	....	.9	8.1	12.2	7.1	880
Alaska	....	....	.0	7.1	20.1	9.1	312
Red Russian	67.8	85.6	49.9	77.4	56.1	61.1	2,118
Fortyfold	73.5	71.2	49.6	76.4	65.2	63.7	479
Hybrid 128	86.6	96.4	63.0	98.1	77.6	79.6	685
Jones Winter Fife	89.3	87.4	78.3	94.2	73.6	82.0	317
Average first 4 varieties	....	....	1.2	6.2	10.8	4.5	....
Average last 4 varieties	79.3	85.2	60.2	86.5	68.1	75.9	....
Average of both resistant and susceptible[b]	....	....	30.7	46.4	39.5	38.9	....

[a] Marquis was spring-sown. All the others were sown in the fall throughout the tests.
[b] See Plates 2, B, and 3, A, for the type of heads of these eight parent varieties.

There is considerable variation from year to year, but the varieties generally maintain their relative positions. The four resistant ones are always comparatively resistant and the susceptible ones are always comparatively susceptible, regardless of seasonal fluctuations. Turkey, representing the extreme resistant type, and Fife, representing the extreme susceptible type, show less variation than the others. Marquis shows less variation than any of the others during the three years recorded and is so uniformly resistant that farmers often take advantage of it and do not treat the seed before planting. It was a matter of considerable surprise, therefore, when it was discovered that Marquis was susceptible when planted in the fall. In 1919 a fall sown row of inoculated seed produced 661 heads, 489, or 74 per cent, of which were bunted. The same phenomenon was observed in 1920, the 14 plants that survived the winter producing 31 per cent bunt, compared with 3.5 per cent produced from a spring planting of 90 plants in the same field. The record of Florence, the other spring wheat, is taken from fall sowings but is somewhat more resistant when spring-sown. Evidently the resistance of Marquis is different from that of the others, inasmuch as it is neutralized by the lowered temperature or the winter rest period. There seems to be no peculiar habit of growth or taxonomic difference associated with this loss in resistance, but it is a constantly recurrent phenomenon obtaining wherever tested. An example of the uniformity of these reactions is shown in the records from Davis, Calif. Seed of the three varieties Turkey, Florence, and Marquis was sent to that Station to be tested for bunt resistance in the season of 1920. Plantings of each were made November 4 and December 21, 1919. More than a thousand heads of each were produced in 1920, of which Marquis contained 37 per cent of bunt heads, Turkey and Florence containing but 7 and 6 per cent, respectively. The spring sowing of Marquis was uniformly resistant.

This does not mean that all the others have the same kind of resistance. In fact they have not, according to the segregation tests of their hybrid progeny, as will be shown later. It seems probable, from a study of Table III, that the different wheats do not react the same to climatic influences from year to year. For example, in 1920 the resistant varieties (with the exception of Marquis) produced more than twice as much bunt as their 3-year average shows, while the four susceptible varieties produced an average of 7.8 per cent less in a similar comparison. The reverse condition obtained in 1916, while in 1918 all varieties produced less than normal, and in 1919 all but Turkey and Alaska produced more than normal. It is very evident that a "good" or "bad" smut year does not apply equally to all varieties.

The percentage of bunt produced on the partly bunted plants was much less on the resistant than on the susceptible varieties, the ratio being 25 to 67 when the four resistant varieties of Table III are compared with the four susceptible ones. This may be taken as an argument in favor of the physico-chemical nature of resistance, for here there can be no question about the plants being infected. The difference is plainly one of greater incompatibility of the resistant hosts. The ratio of total bunt 4.5 to 75.9, however, shows a much stronger contrast and is the just and proper one to use as a quantitative measure of resistance or susceptibility.

SEGREGATION OF HYBRIDS

Only a few of the possible combinations of the eight parent types have been made, but the data obtained are sufficient to show the general type of the inheritance to be expected, and the crosses described below have been tested in sufficient quantities and over a long enough period of time to warrant the conclusion that resistance is definitely heritable according to the commonly recognized laws of genetics. Three types of crosses have been made: resistant × resistant, resistant × susceptible, and susceptible × susceptible. Because a smutted plant is automatically eliminated from further testing, as it produces no progeny, the seed for the F_1 and F_2 generations were not inoculated with spores but were treated with formaldehyde instead, so that both susceptible and resistant segregates were saved for the F_3 generation, which was thus the real beginning of the test. F_1 sibs had been saved, however, so that F_2 sib rows could be tested with the F_3's the same season, under the same conditions. Thus the parent varieties, the F_2's and the F_3's were all tested at the same time in the five crosses that have been carried on in sufficient numbers, and under such conditions as to make the results significant. The amount of bunt produced per row in the segregates of the F_3 generations has been arbitrarily put into 10 groups, each class having an amplitude of 10 per cent. The numbers in each class of 10 crosses is given in Table IV.

TABLE IV.—*Number of F_3 rows falling into each class, when the percentage of bunt produced from 0 to 100 is divided into 10 equal parts*

Variety.[a]	Number of rows falling into class with average percentage of bunt—										Total number of rows.
	5	15	25	35	45	55	65	75	85	95	
A × T	6	1	0	3	0	0	1	1	1	1	14
T × F	101	19	18	12	6	6	3	2	1	0	168
T × H	15	13	9	23	28	33	36	25	11	1	194
T × R	17	9	6	1	4	2	1	2	0	0	42
Fd × T	41	64	37	63	97	104	85	59	38	5	[b] 593
A × J	6	0	0	2	2	0	1	4	9	0	24
Fd × R	1	17	34	55	40	27	5	2	0	0	181
H × M	0	0	0	0	0	0	0	5	53	276	334
A × R	1	0	2	1	0	1	1	2	1	2	11
A × Fd	1	0	1	3	2	1	6	5	1	1	21

[a] T=Turkey, A=Alaska, F=Florence, H=Hybrid 128, Fd=Fortyfold, J=Jones Winter Fife, M=Marquis, R=Red Russian.

[b] From 42 plants selected at random from an F_3 population, which makes these figures really F_4's in which there has been no selective elimination of susceptible segregates in earlier generations.

The average number of plants per row varied in the different crosses from 12.6 to 60.8, and more than 60,000 plants were counted in determining the bunt percentages of the 1,582 rows listed in Table IV. The fact that the tests were made during four different years makes comparison difficult, in one way; yet it tends to add weight to the general conclusions regarding the inheritance of resistance, because of the very fact that the different seasons are included. All of the rows in which Alaska was one of the parents were grown in 1916. The Turkey crosses (except with Alaska and Fortyfold) were tested in 1918. Hybrid 128 × Marquis was tested in 1919, and the other two, Fortyfold × Turkey and

Fortyfold × Red Russian, were tested in 1920. Only the five crosses in which the number of rows exceed 100 will be considered in detail, as the others are too few in number for analysis. The others may be discussed briefly as follows: All four of the crosses in which Alaska was the original male progenitor produced a high degree of sterility, which, combined with lack of winter hardiness, made it difficult to obtain large numbers. The figures as given, however, show that there is segregation, as both resistant and susceptible sorts were obtained in every case. Moreover,

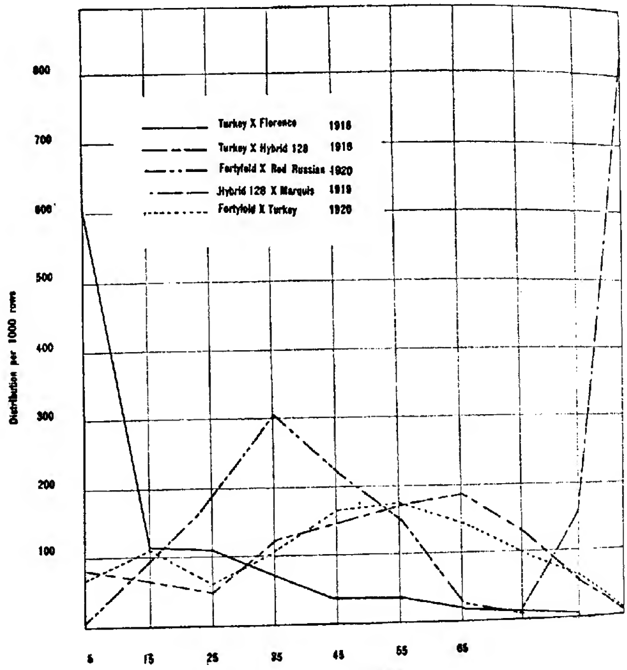

FIG. 1.—Graphical representation of bunt resistance in the F_3 generation of 5 different types of crosses. Three classes of parents were used: Resistant, Turkey (3.5), Florence (7.1); Intermediate, Fortyfold (63.7), Red Russian (61.1); Susceptible, Marquis (74), Hybrid 128 (79.6). Figures in parentheses refer to average percentage of bunt produced under conditions favoring maximum infection.) The crosses of Hybrid 128 with Turkey and Marquis reproduce only the extremes of the parents. In the other crosses transgressive inheritance occurred—that is, segregates were obtained more resistant and more susceptible than the extremes of the parents.

the susceptible classes occur with greater frequency than the resistant ones in the crosses of Alaska and the three susceptible varieties, Jones Winter Fife, Fortyfold, and Red Russian. Crossed with the resistant Turkey, a majority of the F_3 rows are resistant. In fact one of the selections which was sent to the Oregon Station has been found more resistant than either parent there and has proved so promising from a commercial standpoint that it has been continued to the seventh generation. Five F_4 selections and 18 selections in the F_6 generation were grown at Pullman from one F_3 row that was entirely bunt-free, and never a trace of bunt could be found in any later generation, although Turkey

and Alaska, the parent strains, produced a small amount of bunt each year.

An exhaustive test of Turkey × Red Russian was attempted in 1917, but the season was so unfavorable that the grain winter-killed badly and much of it did not germinate till spring, during which time the bunt spores with which the seeds had been inoculated probably perished, for out of 513 rows planted, but 2 produced as much as 10 per cent of bunted heads. Random selection of 42 plants from this F_3 generation gave a preponderance of resistant type in the F_4 generation but was not carried further.

There are five families in which the numbers are sufficiently large and the seasons sufficiently favorable to give results that are dependable for purposes of comparison. The distribution of the different classes has been refigured on the basis of the number of each class per 1,000 rows and the result put in the form of curves in figure 1 in which the ordinates represent the number in each class and the abscissas represent the different classes. There are four distinct types of curves, one in which the 5 per cent class predominates, one in which the 95 per cent class predominates, one in which the 35 per cent class predominates, and two in which the major predominating class is 55 or 65 per cent and a minor class predominating on the resistant end of the curve, and a low center at the 25 per cent class. These two similar crosses are not so similar in reality as they appear from the position of the curves, for the data from which the curve representing the F_3 generation of Turkey × Hybrid 128 was taken was obtained in 1918, while the other curve representing the data of the F_3 generation of Fortyfold × Turkey was obtained in 1920. Reference to Table III shows that the three parent wheats produced an average of 13 per cent more bunt in 1920 than in 1918. If the curves were corrected for this seasonal difference, the F_3 generation of Turkey × Fortyfold would show a parallel curve that would be from 10 to 20 per cent more resistant than the F_3 generation of Hybrid 128 × Turkey. Turkey is one of the parents in both crosses, so the difference in the two curves must be due to the difference in the inheritance of the other two parents, Fortyfold and Hybrid 128. It seems more than coincidence that the 5-year average of the two wheats (Table III) should show 17 per cent less bunt produced by Fortyfold than by Hybrid 128. In fact the F_3 generation of Fortyfold × Turkey shows a mean of 2.3 per cent less bunt than the mean of the other F_3 generation nothwithstanding the more favorable season for bunt in which it was grown. Any combination of the parents in question shows from 10 to 15 per cent more bunt in 1920 than in 1918. The seasonal differences of each parent is, for Turkey, 7 per cent, for Hybrid 128, 14,6 per cent, and for Fortyfold, 15.6 per cent. It seems, therefore, that the curves in question should be separated, on account of seasonal differences, by at least one class, or somewhere between 7 and 15.6 per cent. In other words if the F_3 generation of Turkey × Hybrid 128 had been tested in 1920 instead of 1918 the 194 rows tested would have produced between 7 and 14 per cent more bunt on the average than they did. This would have been between 9.3 and 16.9 per cent more than the F_3 generation of Fortyfold × Turkey actually did produce in 1920. The picture of the inheritance of these two crosses is one in which susceptibility is dominant, with the susceptible segregates fluctuating around the mean of the susceptible parent in each case. A study of the material in the field revealed an important difference

in the two F_3's that the figures in Table IV or the curves in figure 1 do not show clearly, namely, that transgressive segregation occurred in the F_3 generation of Fortyfold × Turkey, there being five rows decidedly more resistant than Turkey and many rows more susceptible than Fortyfold. This phenomenon was not observed in the other F_3 generation, in 1918. Turkey was just as resistant and Hybrid 128 just as susceptible as the extremes of the segregates. This suggests the idea that Fortyfold carries an element of resistance different than the elements of resistance in Turkey, which, added to it, produces a wheat more resistant than Turkey, and without which the segregate becomes as susceptible as Hybrid 128. In susceptible segregates this Fortyfold element of resistance has a value of from 10 to 20 per cent, but in resistant segregates the value lies between 1 and 7 per cent, depending upon the season. It will require tests in later generations to establish this hypothesis beyond question, and this is being done.

It is difficult to place these phenomena of inheritance on a factorial basis on account of the seasonal fluctuations and the quantitative and comparative nature of the material. It is quite evident, nevertheless, that Turkey has several times as much resistance to bunt as Fortyfold. It is also evident, from the performance of the segregates of Turkey × Hybrid 128 in the F_4 and F_5 generations, that this "Turkey resistance" splits up into its component parts when crossed with other wheats of different constitution. Table V shows the comparative resistance of 144 F_4 rows (the offspring of 144 F_3 plants, selected from nine different F_3 rows), in comparison with the parent rows from which they came.

The nine F_3 rows from which the selections were made were the most resistant segregates of beardless, club, winter hardy type (characters which were also being studied). It has been shown in an earlier paper (*14*) that resistance in this cross is not linked with the external characters studied. In comparing the third column, "Bunt in F_3 generation" with the others, it must be borne in mind that these figures were obtained in 1918 and that the F_4 percentages were obtained in 1919, in which year (according to Table III) the parents showed 18.4 per cent more bunt than in the year in which the F_3 generation were tested.

TABLE V.—*Distribution of bunt resistance of Turkey × Hybrid 128 in the F_4 generation from 9 resistant F_3 rows*

F_3 row No.	Number of plants selected from each row.	Average number of F_4 plants per row.	Bunt in F_3 generation.	Average bunt of F_4 rows.	Range of bunt in F_4 rows.	
					Lowest.	Highest.
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
1387	26	71	1.9	12.7	5.6	35.0
1304	22	56	4.8	18.7	6.5	39.6
1310	2	50	6.4	32.0	25.9	38.1
1353	14	66	6.6	13.6	5.6	25.4
1312	29	63	7.0	13.2	4.3	26.7
1284	12	70	7.1	25.3	12.7	40.3
1297	14	42	9.8	33.2	11.6	59.5
1357	8	69	13.4	26.6	16.6	41.7
1299	17	46	14.6	34.8	15.5	54.7
Average	16	59.2	8.0	23.3	11.6	40.1

The season of 1919 was very favorable, and an average of 60 plants per row was obtained. Four of the 9 F_3 segregates produced F_4 progeny almost as resistant as the resistant parent, Turkey. The range of bunt produced by the different F_4 rows indicates that the F_3 row from which they were selected was probably heterozygous for resistance in every case, the range being from 12.2 to 39.2 per cent, which is a larger fluctuation than Turkey. (Ten rows of Turkey were grown in this same series, for comparison, the extremes of variation being 0.7 and 6.9 per cent.) There was, however, not one of the 144 rows in which the plants did not evidence some of the resistance of the Turkey parent, for the susceptible parent, Hybrid 128, growing alongside of the F_4 rows contained 98 per cent of bunt, whereas the worst F_4 row produced less than 60 per cent. The difference was even more outstanding in the field than the figures indicate. Taxonomically the F_4 rows resembled very closely the susceptible Hybrid 128, but the bunt-infected plants were very different. Once a plant of Hybrid 128 became infected it seldom produced any wheat. Out of 125 infected plants but 3 produced wheat, while 25 of the F_4 rows produced some heads of wheat on every one of the infected plants, and only 11 of the 144 F_4 rows contained more than 10 wholly smutted plants.

The plants in 5 of the 29 F_4 rows from F_3 row No. 1312 were superior in yield and quality and were very resistant; they became the progenitors, therefore, of the F_5 selections, five F_4 plants being selected from each row. Five plants were also selected from each of two other F_4 rows for test in the fifth generation. Thus the individuals of 35 F_5 rows representing 3 F_3, and through them 7 F_4 families were tested for bunt resistance in 1920. Ten of these rows—that is, 2 of the F_4 families—were selected for susceptibility instead of resistance to see whether the amount of bunt could be increased by selection after two generations of selection in the opposite direction. The data are summarized in Table VI.

TABLE VI.—*Distribution of bunt resistance in the F_5 generation of Turkey × Hybrid 128 selected from five resistant and two intermediate F_4 families*

F_4 row No.	From F_3 family row No.	Average number of F_5 plants per row.	Bunt in F_4 row.	Average bunt of 5 F_5 rows.	Range of bunt in F_5 rows. Lowest.	Highest.
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
1434	1312	42	4.3	13.3	3.3	22.2
1470	1387	42	7.5	19.4	16.1	22.9
1425	1312	52	7.9	9.1	3.1	15.2
1441	1312	59	8.2	18.3	9.2	35.8
1445	1312	48	8.5	14.7	9.1	18.8
1426	1312	60	27.7	23.2	10.8	37.8
1392	1299	45	37.5	48.3	28.8	67.7

The five plants selected from row 1470 were apparently homozygous for resistance in the F_4 generation but lacked an element of resistance that Turkey possesses, for they varied around the mean 19.4 per cent while Turkey in 1920 varied around the mean 7.6 per cent—the worst year for Turkey since the tests began in 1914. The numbers 1426 and 1392 of Table VI show that they were heterozygous and that susceptibility can be increased by selecting in that direction, for one row was within 10 per cent of being as bad as Hybrid 128.

The total populations of the F_4 and F_5 generations are presented in figure 2 in terms of the distribution of rows in the different classes per thousand, similar to the F_3 distribution in figure 1. It will be seen that the F_4 and F_5 generations very nearly parallel each other. If the two susceptible lines had been eliminated, the other five would show decidedly greater resistance than the F_4 generation in spite of the fact that resistant varieties generally were twice as badly smutted in 1920. (See Table III.) Figure 2 may be taken to fairly represent the first peak of resistant segregates of the F_3 curve in Figure 1. No doubt subsequent selections will isolate the full resistant qualities of Turkey in the progeny in the homozygous condition. It is not unlikely that some of the F_5 rows are already in that condition and can not be changed by selection.

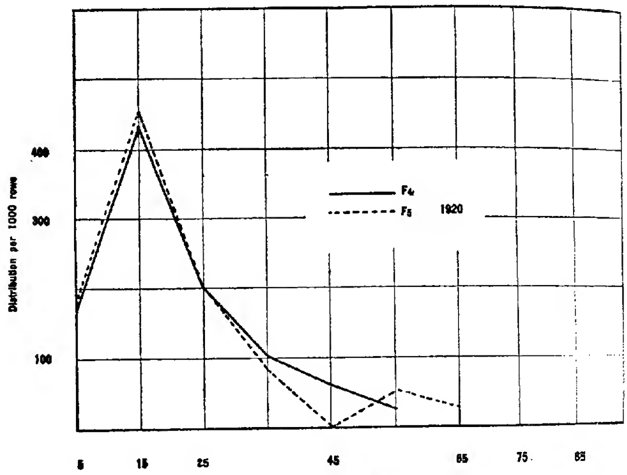

FIG. 2.—Graphical representation of resistant segregates in later generations. The similarity of the F_4 and F_5 segregates indicates the constancy of the selections. The increase on the susceptible end of the F_5 curve is due to the selection of 10 families in that direction from the F_4.

For convenience in summarizing the data in respect to the bunt resistance of Turkey and Hybrid 128 and the progeny of a cross between them, Table VII has been prepared. It should be kept in mind that the result of a given generation represents the constitution of the generation preceding. Thus the results of the F_3 generation show that the F_2 generation reproduced the extremes of the parents with a rather indefinite frequency center near each parent center. The great majority tended toward the center of Hubrid 128, indicating dominance of susceptibility. Similarly the F_4 and F_5 class frequencies indicate the constitutional resistance of the F_3 and F_4 generations. Each later generation shows less variation than the one preceding, as would be expected in a self-fertilized race when selected in the direction of the recessive class center.

TABLE VII.—*Frequency distribution for resistance to bunt in a cross between two varieties of wheat, one resistant, the other susceptible*

Variety.	Year tested.	Bunt in parent row.	Numbers falling into classes with average percentage of bunt per row of—									
			5	15	25	35	45	55	65	75	85	95
		Per cent.										
Turkey	1918	0.5	7	....	....	....	....	....	....	....	....	....
	1919	.6	11	....	....	....	....	....	....	....	....	....
	1920	2.4	5	....	....	....	....	....	....	....	....	....
Hybrid 128	1918	Not tested.	....	....	....	....	1	5	....	2	....	....
	1919	63.0	....	....	....	....	....	....	....	....	....	2
	1920	98.1	....	....	....	....	....	....	1	3	3	....
T×H F_2	1918	Not tested.	....	....	....	1	....	1	....	1	....	....
T×H F_3	1918	do	15	13	9	23	28	33	36	25	11	1
T×H F_4	1919	1.9	12	10	3	1	....	....	....	....	....	....
		4.8	1	12	6	3	....	....	....	....	....	....
		6.4	....	....	1	1	....	....	....	....	....	....
		6.6	3	10	1	....	....	....	....	....	....	....
		7.0	8	16	3	....	....	....	....	....	....	....
		7.1	....	5	3	3	1	....	....	....	....	....
		9.8	....	2	5	2	3	2	....	....	....	....
		13.4	....	4	1	2	1	....	....	....	....	....
		14.6	....	2	6	3	4	2	....	....	....	....
T×H F_5	1920	4.3	1	3	1	....	....	....	....	....	....	....
		7.5	....	3	2	....	....	....	....	....	....	....
		7.9	3	2	....	....	....	....	....	....	....	....
		8.2	1	3	1	....	....	....	....	....	....	....
		27.7	....	2	2	1	....	....	....	....	....	....
		37.5	....	....	1	1	....	2	1	....	....	....

Table VIII gives the class frequencies of Turkey and Florence in respect to bunt resistance as well as those of the progeny obtained from a cross between them. An extra column, not given in Table VII, has been added to show the rows that were entirely without visible infection when harvested.

It will be seen that the F_2 generation (the constitution of which is shown by the F_3 class frequencies) shows transgressive inheritance, with the greatest frequency coinciding with the average resistance of the parents, if it be assumed that the 72 immune segregates varied in the intensity of their resistance in proportion to those in the direction of the susceptible end of the table. Such an assumption seems warrantable from the records of the F_4 and F_5 generations, for they were more resistant than the parents, and only a trace of bunt appeared in each generation in a few of the rows. The F_5 generation shows that 19 out of 25 rows were bunt-free (immune), and their ancestry had been bunt-free since the cross was made. Thus, for three generations these selections have not produced a trace of bunt, although planted each year under conditions favoring maximum infection—that is, the seed was blackened with spores. The parents produced an average of 3.5 and 7.1 per cent under the same conditions, and other varieties, classed as susceptible produced as much as 80 per cent of bunt.

TABLE VIII.—*Frequency distribution for resistance to bunt in a cross between two varieties of Triticum vulgare, both of which are resistant*

Variety.	Year tested.	Bunt in parent row.	Numbers falling into classes with average percentage of bunt per row of—										
			Immune.	5	15	25	35	45	55	65	75	85	95
		Per cent.											
Turkey 326	1918	0.5	1	6	....	....	....	....	....	....	....	....	....
	1919	.6	....	11	1	....	....	....	....	....	....	....	....
	1920	2.4	....	5	....	....	....	....	....	....	....	....	....
Florence 634	1918	.0	1	2	....	....	....	....	....	....	....	....	....
	1919	.9	....	2	....	....	....	....	....	....	....	....	....
	1920	8.1	....	1	....	2	....	....	....	....	....	....	....
326×634 F_2	1918	Not tested.	....	1	1	....	....	....	....	....	....	....	....
326×634 F_3	1918	....do....	72	29	19	18	12	6	6	3	2	1	....
326×634 F_4	1919	.0	11	1	....	....	....	....	....	....	....	....	....
		.0	9	2	....	....	....	....	....	....	....	....	....
		3.2	3	8	....	....	....	....	....	....	....	....	....
		[a] 67.7	3	....	....	....	....	....	....	....	....	....	....
		.0	2	5	....	....	....	....	....	....	....	....	....
326×634 F_5	1920	.0	3	2	....	....	....	....	....	....	....	....	....
		.0	5	....	....	....	....	....	....	....	....	....	....
		.0	4	1	....	....	....	....	....	....	....	....	....
		.0	5	....	....	....	....	....	....	....	....	....	....
		.0	2	3	....	....	....	....	....	....	....	....	....

[a] This is evidently a mistake. The row next to it was immune, and it seems probable that the numbers got mixed at harvest time, for the three selections showed no trace of bunt the following year.

In 1919 an F_3 family of Hybrid 128 × Marquis was produced to test the inheritance of the resistance of Marquis to bunt. Information regarding the inheritance of Hybrid 128 had been obtained the year before from the F_3 generation of the cross between this variety and Turkey. The question arose as to whether the seed should be planted in the fall or spring, one parent being a winter wheat the other a true spring variety. If fall-sown, much winterkilling would be expected, for Marquis is not very winter-hardy. If spring-sown the true winter segregates would not produce heads and two-thirds of the others would be heterozygous for the winter factor and would produce some winter plants and some that would develop late so as to interfere with harvesting operations. The former course was decided upon and the planting was done in October, 1918. The winter was unusually mild, and a good stand was harvested the following summer. Every one was surprised to find all of the F_3 rows (334 in number) very badly bunted. The best row in the lot was three-fourths smutted, and 276 of the rows produced more than 90 per cent of bunt. The whole block of rows appeared as a mass of stinking smut which gave off an offensive odor that penetrated to windward for half a mile. An examination of some demonstration plots in another field showed that the fall-sown Marquis was very smutty. This gave a clue as to why all of the F_3 rows of Hybrid 128 × Marquis were badly bunted. The inherent resistance of Marquis was broken down by the winter rest period.

One plant each from nine of the best rows—that is, those having the least amount of bunt—and one from each of the four worst rows were selected for testing in the F_4 generation. The complete results of the parents, the F_2, F_3, and these F_4 selections are summarized in Table IX.

TABLE IX.—*Frequency distribution of resistance to bunt in a cross between Hybrid 128 and Marquis, both of which are susceptible when fall-sown*

Variety.	Year tested.	Bunt in parent row.	Number falling into class with average percentage of bunt of—									
			5	15	25	35	45	55	65	75	85	95
		Per cent.										
Hybrid 128	1919	63.0										2
	1920	98.1							1	3	3	
Marquis	1919	Not tested.								1		
	1920	74.0				1	[a] 3		[a] 1			
H × M F_2	1920	Not tested.						1				
H × M F_3	1919	do.								5	53	276
H × M F_4	1920	75.9						1				
		76.7									1	
		78.6					1					
		79.4						1				
		80.9								1		
		81.1						1				
		81.3					1					
		83.0								1		
		83.9								1		
		94.2						1				
		96.9								1		
		97.6							1			
		98.4									1	

[a] Seed from a different source, but since Marquis is a variety recently introduced and appears to be constant for all its characters, it is likely that there is no genetic difference in the resistance of these strains, for seed from these same strains planted in the spring produced less than 5 per cent of bunt.

The F_4 generation shows an average of 19.9 per cent less bunt than the F_3 parent rows, but this is undoubtedly due to seasonal fluctuations, for Hybrid 128 and Marquis showed similar differences in 1919 and 1920. A closer study of the F_4's indicates that even in these extremely susceptible segregates there is a constant difference in their susceptibility comparable to the differences in the susceptibility of the parents. This is more clearly brought out by the following figures:

	Average percentage of bunt.		Difference.
	1919	1920	
From 9 low selections	80.2	62.2	18.0
From 4 high selections	96.8	72.4	24.4
Difference between high and low	16.6	10.2	6.4

Out of the 9 low F_3 rows (between 75 and 84 per cent bunt) 5 produced the smallest percentage of the 13 rows in the F_4 generation. Out of the 4 high F_3 rows (between 94 and 99 per cent bunt) selected, one produced the highest amount of bunt in the F_4 generation, and two of the others produced more than the average of all the F_4's in 1920. This indicates that Marquis carries a weak factor or property for resistance that is inherited and is not affected by fall planting. If this be true, strains from this cross could be selected that would show a consistent difference

of from 15 to 30 per cent when planted in the fall under conditions favoring maximum infection. In 1919 Hybrid 128 produced 98 per cent, Marquis produced 75 per cent, and the F_3 varied from 75 to 100 per cent. In 1920 the parent varieties produced 30.9 and 74.3 per cent respectively, an F_2 progeny produced 56 per cent of bunt, and the F_4 selections varied from 46 to 85 per cent. Five plants, each selected from the lowest and highest F_4 row, produced an average of 78 and 91 per cent bunt in the F_5 generation in 1921. These figures include only the rows that were planted together in the same part of the field, and on the same date. There is much work to be done to establish the inheritance of the resistance of Marquis, especially the part played by the winter rest period, the segregation of the units that are not so affected, and the complex of its resistance for spring planting. This is being done and will be reported later.

There remains one other cross to discuss, that of Fortyfold × Red Russian. The average susceptibility of the parent varieties and their F_3 offspring in 1920 was 36.7, 49.3, and 37.5 per cent, respectively. The F_3 segregates varied from 8.1 to 73.6 per cent. Reference to figure 1 shows the greatest class frequency of the 181 F_3 rows to be between 30 and 40 per cent, with rapidly diminishing class frequencies above and below. The most striking thing about this cross is that the average of the F_3 generation is less than the average of the parents. The fact that the parents were duplicated so that there were nearly 200 plants counted precludes the possibility of a chance error. In the other four crosses charted in figure 1 the average of the parents lie below the average susceptibility of their respective F_3's, showing dominance of susceptibility. In this cross there seems to be a slight dominance of resistance, the parents producing on the average 5.6 per cent more bunt than the F_3 progeny.

The lower limit is decidedly lower than any recorded example of either parent under similar conditions. This shows that Fortyfold and Red Russian (which are known to be intermediate in respect to resistance of bunt, when compared with wheats like Turkey, Alaska, and Florence on the resistant side, and Hybrid 128 and Jones Winter Fife on the susceptible side) possess different kinds of resistance which are cumulative in effect, when brought together by crossing. These resistances have a value somewhere between 10 and 20 per cent in reducing the amount of bunt that would be produced on the extremely susceptible varieties when planted under conditions favoring maximum infection. By crossing them, segregates are obtained that are from 10 to 20 per cent more resistant than either parent. It is to be expected, and the evidence points to the probability, that segregates also occur which are from 10 to 20 per cent more susceptible than either parent. The difficulty of isolating the susceptible segregates, on account of the fungus destroying the reproductive organs, was pointed out in another place. The segregable dilute or feeble resistance of Fortyfold shown here is in accord with that found in the cross between it and Turkey. The feeble resistance of Red Russian has not as yet been definitely corroborated in other crosses.

DISCUSSION AND CONCLUSIONS

The investigation described in this paper forms a part of the general work of the Washington Experiment Station looking toward the discovery of methods for reducing the destructiveness of bunt or stinking-smut in

wheat. The particular phase treated is the development of a commercially desirable wheat which will not develop bunted heads even on a badly infected soil. Emphasis has been placed on inheritance of bunt resistance, but the ultimate practical application of the principles involved has been continually kept in mind. (Pl. 3, B.)

The first tests for resistance were made in 1914 when a few of the common commercial varieties were tested for comparative resistance under conditions favoring maximum infection. During the next two years, while these and other varieties were being tested in greater detail, methods of infecting the seed, recording the data, and computing the resistance on a comparative quantitative basis were developed and standardized. In an endeavor to obtain the best possible material for the breeding work, more than 500 named varieties, from the principal wheat districts of the world, including representatives from the eight species, have been tested for resistance.

Most of the varieties tested proved to be either very susceptible or lacking in winter hardiness. Thus the representatives of the four species, einkorn, polish, spelt, and emmer, although all very resistant, were discarded because they were of no commercial value, either from the standpoint of yield or hardiness. One poulard (Alaska) was used as the male parent in several crosses, mainly because of its bunt-resisting qualities. One club (Hybrid 128) was selected for two of the crosses described below because of its stiff straw, early maturity, and prolificacy, notwithstanding its malignant susceptibility to bunt. The other six varieties were selected for the breeding work from the vulgare group because they were common commercial varieties (with the exception of Florence, which was selected for its exceptional resistance) in the Northwest, and contained the greatest range of desirable characteristics.

Alaska, Hybrid 128, Turkey, Fortyfold, Red Russian, and Jones' Winter Fife have the winter habit of growth; that is, they will not head out if planted in the spring, and are considered sufficiently winter hardy for fall sowing. This is of prime importance because the chief losses due to bunt occur in winter wheat. Marquis and Florence are spring wheats and are not considered sufficiently hardy for fall sowing, but were such good milling wheats and so resistant to bunt that they were used for the additional purpose of studying the inheritance of winter hardiness.

These eight varieties (Pl. 2, B; 3, A) have been grown as pure lines from individual plant selections for 6 to 15 years and from the first have bred true for their morphological characters and may be assumed to be constant, within the limits of soil and seasonal fluctuations, in their comparative resistance to bunt.

Turkey is one of the most resistant of all the wheats tested, seldom producing above 5 per cent of bunted heads, even under the most favorable conditions for infection. From one-fourth to one-half of the plants may show infection, but only a few flowers, or at most only part of the heads on an infected plant produce smut balls, the remainder producing normal wheat. The other resistant varieties, Alaska, Florence, and Marquis, show similar characteristics in that only a small part of the flowers on the infected plants are destroyed by the fungus. The two very susceptible varieties, Hybird 128 and Winter Fife, on the other hand, do not normally produce anything but smut balls on the infected plants, the number of flowers or heads escaping being negligible compared with those that are destroyed. An occasional plant escapes infection altogether and produces normal grain; but once the parasite establishes itself within the

host, more than 80 per cent of the plants on the average produce only the fruiting bodies of the parasite, and the others produce less than 40 per cent of grain. Fortyfold and Red Russian, which are intermediate in respect to total bunt produced, are also intermediate in respect to the amount of wheat produced on the infected heads.

The resistance of Turkey is different from that of Alaska, for segregates of a cross betwen them occurred which were more resistant than either parent, inasmuch as no trace of bunt could be obtained from the F_3 to the F_7 generation, although planted under conditions favoring maximum infection—conditions under which both parents showed traces of bunt on more than one-fourth of the plants. Such a result would be impossible unless the resistance of the two wheats were cumulative in effect, each contributing something that the other lacked. For if the resistance were the same in both parents, the offspring would fluctuate around the same mean and no segregates would occur more resistant or more susceptible than the parents. If, however, the resistance of Turkey and Alaska were due to different causes, or perhaps the genes located in different chromosomes, then the offspring would show greater variation than the parents, and segregates would occur which would be permanently more susceptible than either as well as more resistant. If the resistance of each parent were composed of differing multiple factors, many new and different segregates would occur. Thus, if Turkey resistance *abc* met Alaska resistance *xyz*, and each factor reduced the amount of bunt by 20 per cent, a cross between them should give varients (assuming the susceptibility of the parents separately to be 5 per cent) in the F_2 generation that would produce bunt as follows:

Number of resistant factors.	Bunt produced.	Classification.	Number of resistant factors.	Blunt produced.	Classification.
	Per cent.			*Per cent.*	
0	65	Nonresistant.	3	5	Very resistant.
1	45	Slightly resistant.	4	−15 0	Immune.
2	25	Somewhat resistant.	5	−35 0	Do.
			6	−55 0	Do.

Unfortunately the large number of sterile plants and flowers produced by crossing Turkey and Alaska, combined with the lack of winter hardiness, reduced the number of plants to such a degree that an accurate analysis of the particular type of segregation that occurred was impossible.

Florence is a very resistant spring wheat, but its resistance is also different from that of Turkey, for transgressive inheritance occurred in a cross between them, F_3 segregates occurring all the way from completely immune to completely susceptible individuals, not unlike the illustration given above. More than 40 per cent were immune, while the plants in 12 rows (7 per cent of the F_3 progeny) were more than 50 per cent bunt, an amount never produced by either parent under the most favorable conditions for infection. Something like 30 per cent of the rows were intermediate, producing from 10 to 50 per cent of bunted heads.

It is not too much to suppose that the 72 immune rows differed in the intensity of their resistance, but there is no way of proving it except by the slow, laborious process of crossing them with susceptible varieties

and testing the comparative resistance of their descendants. The intermediate and susceptible segregates indicate multiple factors for resistance in both Turkey and Florence, the loss of which renders a given hybrid offspring in a later generation a congenial host for the parasite. Such factors added together are cumulative in effect, making the segregate possessing them immune against all attempts of the organism to set up a pathogenic relationship.

Fortyfold has a dilute or weak resistance that is evidently different from that of any of the resistant elements of Turkey, for in the F_3 generation of Fortyfold × Turkey five out of 593 F_3 families occurred that were distinctly more resistant than Turkey, and many occurred more susceptible than Fortyfold. This dilute resistance of Fortyfold showed a value of approximately 5 per cent in 1920 when added to the resistance of Turkey in the very resistant F_3 families, but in the absence of it and also the Turkey resistance, the segregates increased 15 to 20 per cent in the amount of bunt produced. High and low selections are being tested in the F_4 generation in 1921 to determine the correctness of these assumptions, for the data from the F_3 progeny are not as conclusive as one would like to have in a characteristic which shows such wide fluctuations as bunt resistance.

Hybrid 128 has no heritable resistance distinct from that of Turkey or Marquis, for the F_3 progeny of crosses with them did not give variations exceeding the extremes of the parent types. The intermediates in the F_3 generation of Turkey × Hybrid 128 indicated that the resistance of Turkey was made up of multiple factors, which, when separated, gave dilute resistances. The 334 F_3 families of Hybrid 128 × Marquis were all very smutty, none having less than 70 per cent of bunt. In the F_4 generation, however, the high selections gave 10 per cent more bunt than the low ones, which indicates a dilute resistance, probably of Marquis, that is not affected by the winter weather. Marquis is very resistant when spring sown, but this resistance is mostly destroyed or neutralized by the winter rest period when fall sown.

The resistance of Red Russian is not well established in the cross with Turkey on account of the small numbers and the unfavorable season in which it was tested, but in so far as the F_4 selections tell anything they indicate that any resistance it may possess is not different from that of Turkey, for none of the segregates were more resistant than Turkey, and the most susceptible did not surpass Red Russian in amount of bunt produced. The cross between Fortyfold and Red Russian, however, prove that the latter has a definite heritable resistance which, added to the unlike resistance of Fortyfold, produces segregates much more resistant than either parent. That is, the two weak resistances when brought together have the effect of a strong resistance comparable to that of Turkey or Alaska. The feeble or weak resistance of Fortyfold is in accord with that found in the cross between it and Turkey. The dilute or feeble resistance of Red Russian has not as yet been corroborated in other crosses in sufficient detail to warrant discussion.

From the foregoing evidence, based on seven years' work with bunt resistance in wheat, and with special reference to eight varieties, and the inheritance of resistance in the progeny of crosses between them, the general conclusions may be summarized as follows:

The most susceptible wheats, planted under conditions favoring maximum infection, produce an average of about 80 per cent of bunted

heads. Hybrid 128 and Jones Winter Fife belong to this class. Although they produce 20 per cent of sound heads, this seems to be due to accident, for in crosses with other varieties the descendants do not show evidence of having inherited any cumulative resistance whatever from these varieties.

Fortyfold, Red Russian, and Marquis each have differing dilute resistances which reduce the amount of bunt by 10 to 25 per cent. When added together, as in descendants of crosses between them, the cumulative effect makes a more concentrated resistance, having a value of from 30 to 60 per cent. In addition to the dilute resistance, Marquis has a strong winter-sensitive resistance with a value of 50 to 60 per cent in spring-sown grain which is ineffective in preventing bunt when the seed is fall sown. That is, if Marquis is sown in the fall, only the dilute resistance is operative, for it produces but 10 to 20 per cent less bunt than the most susceptible varieties; if sown in the spring, the strong winter-sensitive factor becomes functional, and the resulting crop produces 60 to 75 per cent less bunt than the completely susceptible varieties. There is some evidence that all the different resistances are somewhat winter-sensitive, for the facultative wheats (those that may be sown either in the fall or in the spring, being true spring wheats but fairly winter hardy) are known to produce more bunt from fall seedings. Florence also registers a higher degree of bunted heads in the fall-sown tests.

Turkey, Florence, and Alaska each have differing concentrated resistances which reduce the amount of bunt 70 to 75 per cent, compared with the standard susceptible varieties. These concentrated resistances are also cumulative in effect when brought together by crossing, the resulting descendants segregating into immune, very resistant, various stages of dilute resistant, and completely susceptible classes.

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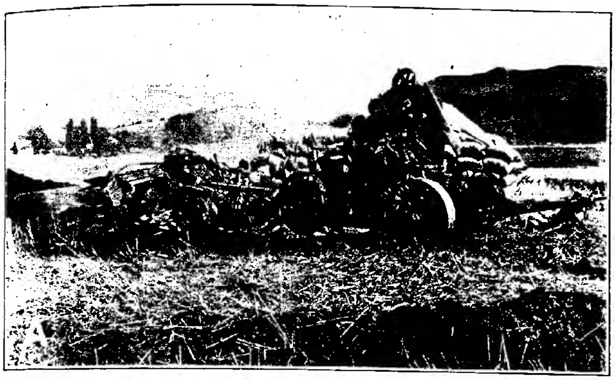
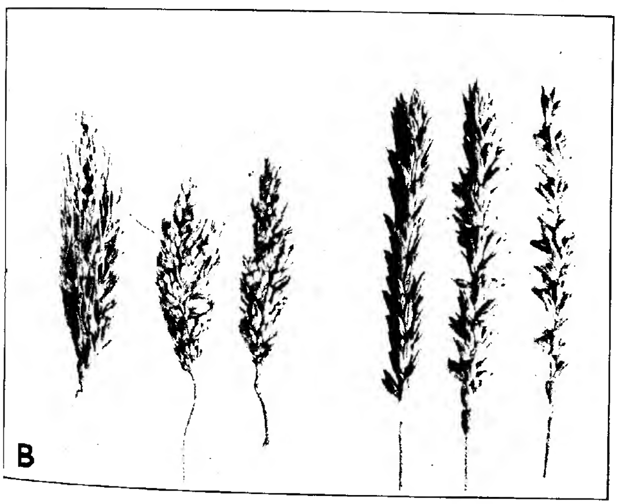
PLATE 1

A.—Result of a smut explosion. Machine of W. J. Grier, near Colfax, Wash., a few hours after the explosion, August 12, 1915. By hard fighting the sacked grain on the right was saved, but the machine and straw stack were burned. Hundreds of thrashing outfits and thousands of bushels of wheat have been destroyed in the Pacific Northwest due to fires following explosions of smut dust (bunt spores), which burn like powder.

B.—Morphological effect of bunt on wheat heads. The three on the left are Alaska, the others Fortyfold. Heads of each variety from left to right contain normal seed, both normal seed and smut balls, and only smut balls. In Alaska the parasite causes the awns to fall but dwarfs the head instead of stimulating increased length as in Turkey and Hybrid 128. (See Pl. 2, A.) The head length of Fortyfold is not affected, but the spikelets on the infected heads stand out more nearly at right angles to the rachis.





B


A


B

PLATE 2

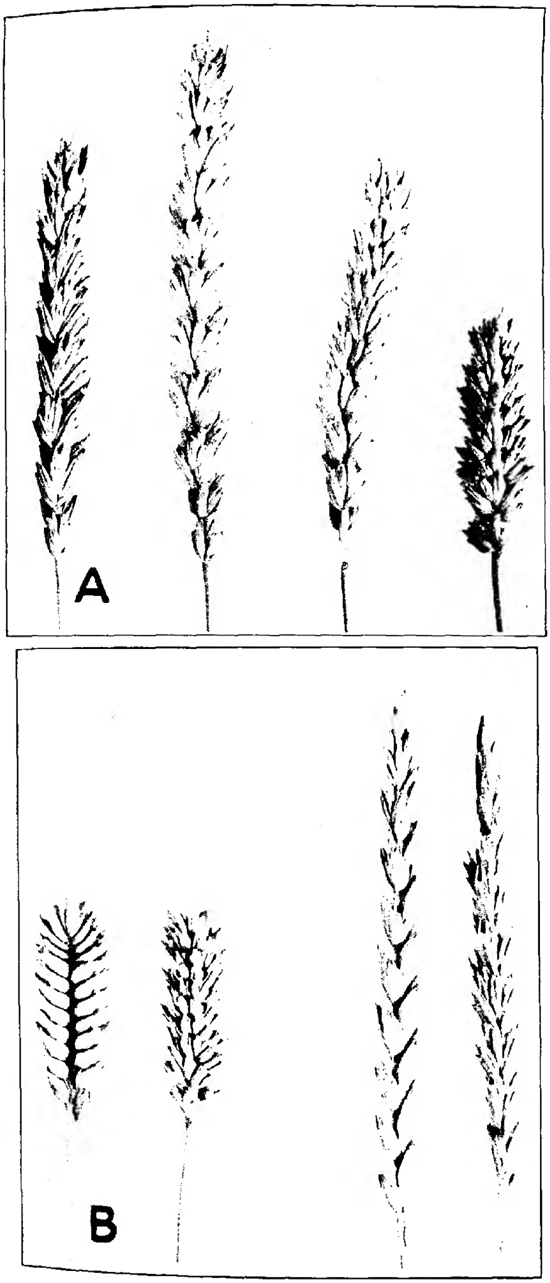
A.—Morphological effect of bunt on wheat heads. The three on the left are Turkey, the others Hybrid 128. First head on the left, normal grain; second head, half wheat and half bunt; third, all bunt. The bunted heads are much elongated as if trying to grow away from the fungus. In Turkey the awns fall off prematurely on the bunted heads. The bunted head of Hybrid 128 on the extreme right looks like a vulgare instead of a compactum.

B.—Parent varieties resistant to bunt. From left to right, Turkey, Florence, Marquis, Alaska. Turkey and Alaska are true winter wheats and can not be used for spring sowing. Florence and Marquis are typical spring wheats. Marquis and Turkey are the most popular varieties in the United States and Canada, measured by the quantity produced. Both are excellent breadmaking wheats.

PLATE 3

A.—Parent varieties susceptible to bunt. From left to right, Fortyfold, Jones Winter Fife, Red Russian, Hybrid 128. Jones Winter Fife and Hybrid 128 are about 20 per cent more susceptible than the other two. All are true winter wheats of commercial importance in the Northwest. Fortyfold and Hybrid 128 have white grain.

B.—Two hybrid (F_2) types with commercial possibilities. Left, the morphological characters of the Hybrid 128 parent are combined with the resistance of Turkey. On the right the Florence head type is combined with the winter hardiness of Turkey. It has been immune to bunt for three years. Both selections outyielded the parents in 1919 and 1920.



A BACTERIAL LEAFSPOT OF TOBACCO[1]

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INTRODUCTION

During the past five years a bacterial leafspot of tobacco occuring in Wisconsin has been the subject of investigation together with observations on tobacco leafspots in general in various tobacco districts of the United States. The tobacco leaf is subject to an exceptionally large number of diseases, originating from a variety of causes, in some cases with distinctive and constant symptoms, but more often confusing when any determination or classification based on symptoms is attempted. This confusion is in some respects magnified by the fragmentary early literature and is due largely to common names having been applied without adequate consideration as to the causal agents of the diseases. Recently two or three leaf diseases have been described, however, in sufficient detail to permit of definite reference. It is the purpose of this paper to contribute to the knowledge of one other such disease.

CAUSE

This disease, which is not believed to be new to tobacco growers in Wisconsin, has been found to be due to a bacterial organism apparently previously undescribed. The causal organism has been named *Bacterium melleum*, n. sp., and a description of the organism and the symptoms of the disease it causes is given in this paper.

COMMON NAMES

The leafspot to be described here is ordinarily called "rust" by Wisconsin tobacco growers. This name, which is not a good one from a phytopathological standpoint, is in fact in use throughout most of the tobacco districts of the world, for a variety of leaf diseases of tobacco, and is, therefore, a very unreliable term. The term "rust" is frequently limited by the use of such combinations as "red," "brown," "white," and "black rust;" but these again are of little significance when applied by different individuals and have little definite relation to the causal agent concerned. Other terms such as "firing," "black fire," field fire," "wildfire," "speck," and "frogeye" are terms which have been commonly used as synonymous with "rust," though the term "frogeye" is now generally limited to the disease caused by *Cercospora nicotianae*

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El. and Ev. and more recently "wildfire" has become the accepted common name for the disease caused by *Bacterium tabacum* Wolf and Foster.

The leafspot diseases of tobacco naturally fall into three disease categories, as regards cause, namely, (1) those due to nonparasitic agents, (2) those due to fungi, and (3) those due to bacteria. It is probable that additional terms should not be added to the list of common names until a satisfactory basis of classification based on these categories is established. However, we have come to refer in the laboratory to the disease herein described as the "Wisconsin bacterial leafspot" disease, and this term may tentatively be preferable to the commonly used term "rust."

OTHER BACTERIAL LEAFSPOTS

Although it is only within recent years that certain leafspots of tobacco have been definitely shown to be of bacterial origin, it is fairly certain that one or more have existed from the earliest days of tobacco culture in this country. The earliest treatises on tobacco culture refer to "rust" and "firing," although in most cases it would be difficult to judge the nature of the causative agent from the descriptions of these diseases. Killebrew and Myrick (*6*),[2] for instance, wrote as follows—

> another field fire, called "black fire," which is totally different from the red field fire, is caused by excessive humidity and occurs only after continued rains of several days' duration with hot weather. This black fire is much more to be dreaded than the brown rust or red field fire, for it attacks the plants while *immature*, involving all the leaves. Sometimes the disease will spread over a field in two or three days and ruin the crop, making black deadened spots as large as a silver dollar, but this rarely happens.

This disease was undoubtedly parasitic in nature, especially in view of the fact that these experienced observers separated it from other symptoms probably of nonparasitic origin. It is also quite likely that this disease was one of the two tobacco leafspot diseases recently shown to be of bacterial origin in this country.

Similarly the disease described in this paper as "Wisconsin leafspot" has probably existed in Wisconsin for 50 years or more along with other leafspots under the name of "rust," but now referred to by some of the older growers as "old-fashioned rust," on account of the fact that it has not been as prevalent in recent years as in the earlier days of the industry in this State.

Apparently the first leafspot disease of tobacco attributed to bacteria was that of "la rouille blanche" (white rust) of France, ascribed by Delacroix (*1*) in 1905 to *Bacillus maculicola*. The description of this disease is not sufficient to afford adequate comparison with our American leafspots, but in any case the description given indicates that it is different from the Wisconsin bacterial leafspot. Honing (*4*) in 1914 described a rust occurring in Deli (Sumatra) which he showed to be due to a bacterial organism which he named *Bacterium pseudozoogloeae*. This disease was also known as "black rust," although it evidently was not the "wildfire" disease of America, since neither the description of the causal organism nor that of the symptoms of the disease correspond with that of wildfire. Honing's disease corresponds more closely to that of the Wisconsin leafspot, although, as will be shown by later comparison, they differ in several respects.

[2] Reference is made by number (italic) to "Literature cited," p. 492–493.

Wolf and Foster (7) in 1917 described the wildfire disease as it occurred in North Carolina and Virginia, following an unusually severe outbreak and proved the causal organism to be a bacterium, which they named *Bacterium tabacum*. This disease apparently has since spread to most of the other tobacco districts in the United States, seemingly from the North Carolina epidemic of 1917 as a center of infection. This disease is not readily distinguished from the Wisconsin leafspot in general symptoms, although the chlorotic area around the point of infection is usually larger and more common than in the Wisconsin leafspot. The wildfire organism is also a much more vigorous parasite than the Wisconsin leafspot organism, and the disease may consequently be much more prevalent and serious where it occurs. Fromme and Murray (3) investigated a leafspot disease in Virginia, which had apparently also existed for a considerable time in that State, and found it to be due to a bacterium which they named *Bacterium angulatum*. This disease has been named by them "angular leafspot" on account of the angular shape of the lesions. It cannot be readily confused with other bacterial leafspots but may be difficult to distinguish at times from certain nonparasitic spots when judged by symptoms alone.

DESCRIPTION OF THE DISEASE

The Wisconsin bacterial leafspot has been found ordinarily on the lower leaves of the plants in the field. Usually it is most marked on the lowest leaves but has been observed during this investigation up to the middle leaves. In severe outbreaks, not seen since the beginning of this study but earlier noted and referred to by others as "old-fashioned rust," the leaves on the entire plant may be involved. That the disease may occur on young leaves is evidenced, however, by the observation of several infections in seed beds from which the causal organism has been isolated. The older leaves are seemingly more predisposed to a rapid collapse and death, and finally, browning of the tissue when once infected, but when artificially inoculated by needle punctures the top leaves show equal predisposition to infection, and the development of the chlorotic area surrounding the point of infection is even more marked than on lower leaves. The common occurrence of the disease on the lower leaves in the field is due quite likely to the more favorable environmental conditions offered there for infection and progress of the disease.

The disease in the seed beds ordinarily is inconspicuous and not as typical as in the field. The spots are usually small and more angular than in the field and the chlorotic area less distinct (Pl. 2, A). The old lesions are usually small and light-colored, but when they run together the young leaves present the appearance of being blighted.

In the field the young spots are usually round, frequently with a small central fleck surrounded by a distinct chlorotic area or halo, identical with that of the wildfire disease. Under other conditions this halo may not appear at all or may disappear rapidly, the tissue surrounding the point of infection collapsing and soon turning brown in color, in some cases possibly white. Enlarging spots may or may not be limited by the veins of the leaf. At times the infected area seems to pass over the vein without injury to it. At other times, however, the parasite may enter the vein and follow it, producing an elongated lesion. The diameter of the spots may vary from 1 mm. to 1 cm. or more, frequently coalescing, and hence involving large areas of the leaf. The old lesions

are usually distinctly brown in color, sometimes brownish white, frequently with a dark center giving a "birds-eye" appearance. Concentric rings, usually are not present, though apparently they may occur (Pl. 1, A, B).

An interesting symptom frequently evident in the greenhouse after artificial infection is the formation of a secondary ring of small lesions 2 or 3 mm. beyond the circumference of the primary lesion. This ring, often perfect in shape, seems to follow as a result of renewed activity by the parasite about the primary lesion following a checking of the disease. The chlorotic area surrounding the center of infection has been found to be relatively free of organisms, as was found by Miss Elliott (2) in the halo-blight of oats.

PREVALENCE OF THE DISEASE

The causal organism of the Wisconsin leafspot disease was first isolated in the spring of 1917. On account of the similarity of other leafspots, nothing conclusive can be said as to the prevalence of the disease prior to that time, although the writer feels confident that within 20 years of casual observation previous to 1917 he has seen a number of more serious cases of the disease than have been noted since. This belief is strengthened by the testimony of a number of the older tobacco growers in the State, who recall complete losses of portions of crops from "rust," which, from our subsequent observations on nonparasitic leafspots, are not believed to develop to such an extent on the type of tobacco grown in this State, with the possible exception of the "rust" following mosaic. "Rust" following as a result of mosaic is not, however, ordinarily limited to such an extent by the topography of the land and the opportunity for infection as is the bacterial leafspot. Since 1917 a number of mild occurrences of the disease have been seen within a 25-mile radius of Madison, and in most cases have been identified by isolation of the causal organism and inoculation experiments. Search has been made for this disease in a number of other tobacco districts, mostly in Kentucky, Maryland, Pennsylvania, and in the Connecticut Valley. Only one specimen has been collected which can with certainty be said to be the same disease, and this was from Kentucky in 1919. One collection from Connecticut in July, 1919, proved to be the "wildfire" disease, and was the first record of that disease in the Connecticut Valley. Similarly, collections made in 1920 from Maryland, Kentucky, and Ohio proved to be wildfire.

ISOLATIONS

The first isolation of the Wisconsin leafspot organism was made in June, 1917, from the seed beds at the experiment station at Madison. At about the same time specimens of a leafspot on tobacco seedlings (wildfire) were received from Mr. E. G. Moss, in charge of the branch tobacco station, Oxford, N. C. This disease was at first thought to be due to a fungus, and preliminary isolation and infection experiments were conducted from this standpoint with negative results. Bacteria were soon after isolated and infection secured. Word was then received that Dr. Frederick A. Wolf, of the North Carolina Station who was working on the same disease, had established the bacterial relationship, no doubt a few days before our own conclusion had been reached. A few weeks later the writer visited the North Carolina section and had the opportunity of noting a second serious outbreak of the disease in that section.

On his return to Wisconsin similar leafspots were noted, among them certain spots on a row of a southern type of tobacco in the experimental plots at Madison, though not occurring nearly as seriously as in the fields seen in North Carolina. The records show that two of the isolations from this row gave white organisms, one of which was infectious. Unfortunately this culture died before a detailed study could be made of it, so that we are not at all certain that it was the wildfire organism. The writer's earlier isolation from the seed bed leafspots and later isolations from the field yielded, however, only yellow infectious organisms. No white organism has since been isolated except following known cases of inoculation with the wildfire organism. It is felt that this explanation should be made here in view of a statement made by Wolf and Foster (7) as a result of correspondence, to the effect that wildfire occurred in Wisconsin in 1917. While the "similar spot" referred to has developed to be what we now call the "Wisconsin leafspot," there is still some probability that we did have one case of wildfire on a row of southern tobacco in 1917, and if so, it seems likely that it was the result of seed-borne infection. In any case wildfire can not be said to have occurred in Wisconsin in 1917 in the sense that it has since been reported from other States, nor was it introduced in that manner until 1922.

During the last five years a large number of isolation tests have been made from various sorts of leafspots of tobacco. Wherever fairly fresh and young spots of the wildfire disease or the Wisconsin leafspot have been plated out, no difficulty has been encountered in getting pure cultures at once, so that the distinction between these two diseases has been readily established. The method employed has been simply to select a young lesion, cut it out with a scalpel, and rinse it through 8 to 10 sterile water blanks with vigorous shaking. It was then transferred to a tube of bouillon, mashed with a sterile scalpel or rod on the side of the tube, rinsed into the bouillon and allowed to stand 15 to 30 minutes. One to six loopfuls of the bouillon werethen transferred to melted potato agar at about 45° C. and plates poured. Cultures were usually kept in stock on potato-dextrose agar in the ice box. Twelve different isolations of the Wisconsin leafspot organisms have been made over a period of five years. Practically all the cultural character studies were made with one organism (culture No. 141), and where this culture was not used we have made sure that we have used a pathogenic organism corresponding in ordinary cultural characteristics.

INOCULATION EXPERIMENT

A very considerable number of inoculation experiments have been made in connection with demonstrating the pathogenicity of the organism isolated, testing the strains following growth in culture for different periods, and for comparison with other bacterial leafspots of tobacco, particularly wildfire. Repeated trials have also been made comparing inoculation by spraying and by needle puncture, under very variable environmental conditions. It has been found that, while good infection is always secured on wounded leaves with a virulent strain of the Wisconsin leafspot organism, practically no infection at all has ever been secured by simply spraying the plants with a suspension of the organism in water.

The writer can not state with certainty the relation of normal field infection to wounded tissue. He has not been able to find from observa-

tion that wounding by insects or other means has played any part in infection. It seemed rather that infection in the field was dependent upon the occurrence of favorable environmental conditions. Every attempt to duplicate such conditions experimentally has thus far given negative results. The writer has as yet, however, done nothing as regards the intrinsic predisposition of the plant itself to infection, and it is not improbable that the host grown under different conditions as regards chemical and physical relationships may be considerably altered thereby as regards predispostion. This belief is strengthened by the fact that similar experience has been had with frequent attempts at securing good infection with the wildfire organism by spraying under greenhouse conditions, while with this organism we know that under other conditions good infection may be secured in this manner.

The method of inoculation by wounding has been essentially that of puncturing the leaf with a needle point which has been dipped in a suspension of the organism in water and permitting a small droplet of the watery suspension to cover the wound. In this manner, when a virulent strain of Wisconsin leafspot organism is used, infection is secured in two to five days, and symptoms develop which compare favorably with those from *Bacterium tabacum* in size of chlorotic area or lesions obtained (Pl. 2, B). On the other hand, it is certain that under field conditions the Wisconsin leafspot organism is not as virulent as the wildfire organism, and that the former can be pathogenic only under more limited conditions than is the latter. Under field conditions the chlorotic area or halo formed by the Wisconsin leafspot organism is not normally as marked as that of wildfire (Pl. 1, A) and frequently may not occur at all (Pl. 3, A) on older leaves, where conditions are seemingly more favorable for a rapid collapse of the leaf tissue than is the case on the younger leaves.

Considerable uncertainty has been experienced throughout the progress of this investigation as to the continued pathogenicity of the organisms carried in culture on potato-dextrose agar, and as a result frequent inoculations have been made to test this point with various cultures. In large measure, the same has been true of the wildfire organisms carried along simultaneously.

A large number of subcultures have died, lost their pathogenicity completely, or in considerable part (Pl. 2, B).

This has been due apparently in most cases to an unfavorable cultural medium, although in many cases this occurred on potato-dextrose agar made according to the same formula as other batches in which organisms have been kept alive and virulent through transfers kept in the refrigerator for three years or more.

A limited number of inoculations have been made upon other host plants aside from ordinary tobacco (*Nicotiana tabacum*). Infection has been secured upon various other species of Nicotiana, especially *N. glauca* and *N. rustica*, and also upon the tomato, together with some indication of infection upon certain cereals.

CULTURAL CHARACTERS

Morphology.—The organism is a short motile rod with rounded ends, occurring singly, in pairs, or occassionally in short chains. Measurements under various conditions have ranged from 0.5 to 0.8 microns in width by 1 to 2.4 microns in length, averaging about 0.6 by 1.8.

Stained by the Caesar-Gil and modified Pittfield methods for flagella from 24-hour-old cultures, the organism shows from one to several polar flagella, usually two or three, but as many as seven have been counted. The flagella are ordinarily from three to five times as long as the organism itself. The organism has been stained with carbol fuchsin, methylene blue, and anilin gentian violet. No spores or involution forms have been observed. Capsules are formed. Pseudozoogloeae are apparently absent. It is Gram-negative and is not acid-fast.

POTATO-DEXTROSE AGAR.—Most of the cultural work has been done on potato-dextrose agar, as this had been found to be most useful for rapid comparative purposes on account of the color imparted to the agar. Colonies in agar plates are first visible in about 36 hours at about 22° to 26° C., increasing to 3 to 5 mm. in diameter in six days. Colonies are at first grayish white, changing on most media on about the third day to a distinct yellow, after which a transparent light yellow tinge develops in the potato agar. The colonies are round, shining, convex, and yellow with opaque centers. The submerged colonies are lenticular. On agar slants a distinct growth may appear along the line of inoculation in 24 hours, and this broadens gradually at the base, becoming echinulate (Pl. 4 A). The growth becomes fairly heavy in 3 to 5 days but rarely covers the slant, usually developing a fairly deep orange color. Certain potato-dextrose agar media will color up in a few days, but ordinarily more gradual coloration occurs, the media becoming usually a bright honey yellow, which may extend to a depth of 2 inches or more from the growth down into the tube. Where the pigment is rapidly absorbed by the agar, the growth does not take on a deep color. Most of the yellow bacterial plant pathogenes known to science have been cultivated simultaneously, but with none has this charactristic of yellowing potato-dextrose agar been nearly as distinct, and with the majority seemingly it does not occur. The pigment on potato-dextrose agar is soluble in water, sulphurous acid, ammonia, methyl and ethyl alcohols, and hydrogen peroxid. It is apparently destroyed by hydrochloric acid, toluène, xylol, benzol, and carbon disulphid. Its production is slowed up markedly at 10° C., but between 20° and 33° it is normal. Light apparently does not influence its production. Its intensity varies considerably on different potato agars, which may possibly be due to differences in reaction of the media.

GELATIN.—Gelatin is rapidly liquefied in thickly sown plates, usually within 48 hours. In gelatin stabs liquefaction usually begins in 48 hours (assumes a stratiform shape), and may require two to three weeks for completion.

NUTRIENT BROTH.—Decided clouding occurs in 24 hours in beef-peptone broth (+10 Fuller's scale) with moderate flaky sediment. On slight shaking the sediment readily breaks up into a fine suspension. Cloudiness does not appreciably increase after 3 days. A very thin surface membrane may be formed, after several days, but this characteristic is not marked.

POTATO CYLINDERS.—Good growth in 48 hours, of brownish yellow color. Growth is profuse in 5 days, with increasing yellowing of organism along line of streak and darkening of medium. Tests with iodin indicate marked conversion of starch to amylodextrin, but diastatic action on the whole is feeble as compared with *Bacterium campestre*.

LITMUS MILK.—The color becomes more intense on the second day, with the formation of a thin blue layer in the upper portion of the medium which disappears about the third day. After 4 days the casein is precipitated, and in 6 more days the clearing has proceeded two-thirds of the way down the tube. The liquid is dark green on top and shades down to a tan just above the casein. The medium on long standing finally becomes a deep blue-green.

COHN'S SOLUTION.—Marked clouding occurs in 2 days or less, followed in about 4 days by the appearance of a heavy white crystalline membrane on the surface and a faint greenish tinge below it. On long standing the medium becomes light yellow in color and contains a flocculent precipitate.

FERMI'S SOLUTION.—Clouding becomes very marked after 2 days. After about 8 days the medium takes on a light greenish tinge, but this is not as marked as with *Bacterium tabacum*. After about 10 days or more a fairly heavy membrane is formed and the sediment increases. On longer standing the medium turns to an intense honey-yellow color.

BEEF AGAR STROKE.—On beef agar slants growth is distinct in 24 hours, grayish white in color, turning finally to a deeper yellow. Growth less profuse than on potato agar and no coloring of medium evident.

LOEFFLER'S BLOOD SERUM.—Growth grayish yellow, spreading, resulting in gradual liquefaction of medium.

NITRATE IN NITRATE BROTH.—There was no reduction of nitrates in nitrate peptone broth.

INDOL, AMMONIA, AND HYDROGEN SULPHID.—Negative tests for all by usual methods.

FERMENTATION TESTS.—From a 2 per cent Difco peptone solution five different carbon media were made by adding 1 per cent of the following: Saccharose, dextrose, lactose, glycerine, and dextrin. In fermentation tubes no gas was formed with any of these compounds. Distinct clouding appeared in the open arms in 48 hours. In the case of saccharose and dextrose slow clouding also appeared in the closed arms. No acid was produced in any of the tubes.

LITMUS SUGAR AGARS.—Tests with lactose, glycerine, saccharose, dextrose, dextrin, agars showed no formation of acid in any case.

TOLERATION OF ACIDS AND SODIUM CHLORID.—No growth was obtained in tubes of neutral beef-peptone broth to which 0.3 per cent of malic, citric, or tartaric acid had been added (P_H values 3.6 to 4.0). A concentration of 0.2 per cent of malic and tartaric limited growth but 0.2 citric did not. Two to 3 per cent sodium chlorid limited growth markedly and 4 per cent inhibited growth entirely.

OPTIMUM REACTION AND TOLERATION LIMITS.—The best growth in beef-peptone broth was secured at + 10 to + 15 Fuller's scale. The maximum for growth lies apparently close to + 20, + 22 giving no growth. While good growth was secured in some instances as low as −20 it is not believed that these results are significant, since the broth after adjustment and standing for some time usually rose to −4 or higher.

TEMPERATURE RELATIONS.—The optimum for growth in culture lies close to 26° to 28° C. No growth was secured at 35° to 36° or below 7° to 9°. The thermal death point found by subjecting freshly inoculated tubes of boullion to different temperatures for 10 minutes was found to be about 57°.

RESISTANCE TO DESICCATION.—The organism dried on cover slips in sterile Petri dishes did not lose its power of growth until after 14 days.

RELATION TO OXYGEN.—No growth in atmosphere freed from oxygen by pyrogallol-KOH method. Some growth in closed arm of fermentation tubes with saccarose and dextrose.

EFFECT OF SUNLIGHT.—Fifteen minutes' exposure of plates on ice to sunlight killed a few organisms, and practically all were killed on 30 to 60 minutes' exposure.

VITALITY AND VIRULENCE.—The organism can be kept in culture and maintain its virulence for at least three years. It may lose its virulence, however, upon certain media while still giving normal growth in culture or it may die out rapidly on presumably favorable cultural media for reasons not definitely understood.

GROUP NUMBER.—221.3333633. The name *Bacterium melleum*, n. sp., is suggested for this organism, basing the specific name on the honeylike color imparted to potato dextrose agar.

TECHNICAL DESCRIPTION

Bacterium melleum, n. sp.[3]

A short rod with rounded ends, occurring singly, in pairs, or in chains. Average size 0.6 by 1.8 microns. Motile by a tuft of polar flagella usually two to three in number, but ranging from one to seven, and three to five times as long as the body of the organism. No spores or involution forms have been observed. Capsules are present. It is Gram-negative and not acid-fast. The organism is pale or orange yellow on most media, particularly on potato-dextrose agar, to which it imparts a honeylike pigment. Growth on potatoe agar stroke is abundant, echinulate, raised, glistening, smooth, and viscid; agar colonies grow rapidly, are circular, smooth, and convex. On nutrient broth the surface growth is slight or none, clouding strong, and sediment somewhat flaky, more amorphous, and moderate in amount. In gelatin stabs growth is best at top with liquefaction, beginning in 3 days and complete in about 20 days. The coagulation of milk is prompt, coagulum slowly peptonized. Alkaline reaction with litmus milk, with prompt reduction. Good growth in Fermi's and Cohn's solution. No indol or ammonia produced. Nitrate in nitrate broth not reduced. Optimum temperature for growth about 26° to 28° C., maximum 35° to 36°. Thermal death point 57°. Optimum reaction for growth in broth +10 Fuller's scale. Pathogenic on *Nicotiana tabacum*, causing a leafspot with or without a chlorotic halo around the point of infection, usually followed by browning of affected tissue.

COMPARISON WITH OTHER BACTERIAL LEAFSPOTS OF TOBACCO

It is evident from the description of the causal organism that the Wisconsin leafspot differs from that of the previously described American tobacco leafspots, namely, wildfire and angular leafspot. The most striking difference is that of the color of the pathogenes, which is white in both of the latter diseases but yellow in the Wisconsin leafspot. As far as symptoms themselves are concerned, one could not with certainty distinguish between wildfire and Wisconsin leafspot, though the former is the more destructive and widespread and under field conditions usually possesses the most marked chlorotic halo.

The Wisconsin leafspot disease is not so readily distinguishable from the Sumatran disease described as black rust by Honing (4). Neither specimens of this disease nor the causal organism have been seen. Therefore Honing's description offers the only basis for comparison.

[3] According to the classification of the American Society of Bacteriologists the combination would be *Psuedomonas mellea* n. sp.

The illustration of black rust by Honing shows some resemblance to Wisconsin leafspot, but the disease does not seem to be identical with the latter. The chief points of difference between the two causal organisms may be summarized as follows:

Bacterium pseudozoogloeae (Honing)	*Bacterium melleum*, n. sp.
1. Produces "black rust."	1. Produces "brown rust."
2. Apparently no chlorotic halo.	2. Chlorotic halo frequently present.
3. Produces lesions with concentric rings.	3. Lesions usually not concentrically ringed.
4. Size generally 1.5 microns by 0.7 to 1 micron.	4. Generally 1.8 microns by 0.6 micron.
5. 1 to 2 polar flagella.	5. 1 to 7 polar flagella.
6. Color usually yellowish gray.	6. Color usually orange-yellow.
7. Gelatin stab papillate; liquefaction napiform to saccate.	7. Gelatin stab filiform; liquefaction stratiform.
8. Milk coagulum not peptonized.	8. Milk coagulum peptonized.
9. Litmus milk rendered acid.	9. Litmus milk rendered alkaline.
10. Acid with dextrose, lactose, and saccharose broth.	10. No acid with dextrose, lactose, and saccharose broth.
11. Fluorescence yellowish green (in gelatin).	11. Fluorescence honey-yellow on potato-dextrose agar.

From this comparison it may be seen that the differences are decided in many instances and that the likelihood of the Sumatran and American leafspot being identical is very remote.

PRACTICAL CONSIDERATIONS

The Wisconsin bacterial leafspot or "rust" no doubt occurs annually in this State, or at least it has been found for the last five years without much difficulty, although not to such an extent as to cause much concern. As already stated, however, it is quite certain that in years past it has been the cause of considerable losses and the object of demand for control measures. This belief is strengthened by the writer's recent studies of nonparasitic spotting of tobacco, which might otherwise have been confused with the bacterial leafrust. The causal organism is not believed to be a vigorous parasite, and special conditions are necessary for infection without wounding. Aside from a period of rainy or humid weather, and possibly a fairly high temperature, we do not know the conditions which are necessary for infection, since these two requirements in themselves are apparently not sufficient. This conclusion is arrived at as the result of environmental studies in controlled temperature and humidity chambers.

There is some ground for the belief that plants may be predisposed to the disease from internal causes. This hypothesis may be illustrated by an observation of the behavior of this disease in field fertilizer plots at the Wisconsin Experiment Station. The plots concerned were in duplicate and were intended to compare the value of barnyard manure with commercial fertilizers. For some reason not clearly understood, the manured plots gave a yield considerably lower than the unfertilized plots. Counts were made of the number of infected plants in each plot and, as may be seen from Table I, a fairly close correlation existed between yield, or the fertilizer applied, and the amount of infection.

TABLE I.—*Percentage of "rust" on fertilizer test plots*

Plot No.	Fertilizer applied per acre.	Yield in pounds of cured leaf per acre.	Percentage of plants rusted.
3	Barnyard manure, 20 tons	1, 545	43
8	....do	1, 242	46
	Average	1, 393	44.5
1	No fertilizer	1, 682	23
7	....do	1, 527	26
	Average	1, 604	24.5
2	200 pounds nitrate of soda, 200 pounds sulphate of potash, 600 pounds acid phosphate.	1, 695	11
6	....do	1, 735	10
	Average	1, 715	10.5

It does not follow from this, however, that "rust" is more likely to occur in low-yielding crops, the manured plots in this test being in fact an average crop. The condition is rather one of "physiological or nutritional balance" in the plant, a condition, as yet very inadequately understood in relation to plant diseases. A practical suggestion for experimental work in reducing damage from this disease by proper fertilization is offered, however, by such observations.

It has usually been found that the first or primary infection starts in the seed bed and that the secondary infection in the field is a direct result of transplanting infected plants. The same seems to be especially true of the wildfire disease. In the springs of 1917 and 1918 infection with Wisconsin leafspot was first noted in the seed beds at the Wisconsin Agricultural Experiment Station, and subsequently secondary infection in the field was found in areas in the field planted from the infected areas in the seed beds, although a considerable time intervened between the two appearances of the disease, during which time it had apparently disappeared. This experience, together with a similar, and now common, experience with the wildfire disease, offers the suggestion that seedling plants from areas showing signs of infection should preferably not be transplanted into the field. It is not improbable that spraying with copper sprays in the seed beds as suggested for wildfire (5) may also help to control the Wisconsin bacterial leafspot should conditions warrant its use.

The manner in which the disease lives over winter is not definitely known. The causal organism may perhaps live over on the seed, on the cloth covers, possibly in the soil, or by other means. Until this is determined no satisfactory means of control from this standpoint can be offered.

Although a considerable number of varieties of tobacco have been grown at the Wisconsin station annually, these have not all been equally exposed to infection. From limited observations and experiments, however, the writer feels safe in concluding that differences in varietal susceptibility or resistance are small, if in fact they exist at all.

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SUMMARY

A bacterial leafspot disease of tobacco has been under observation and study in Wisconsin for five years. This disease is one of three or more different leafspots of tobacco commonly grouped under the common name "rust" by the growers in Wisconsin. The other "rust" spots appear to be nonparasitic in nature.

This disease has not been especially serious in recent years, but it is believed that it is the "rust" which has been most serious in past years and may become so again at any time that the required favorable conditions for its occurrence appear.

The Wisconsin leafspot of tobacco differs from both the wildfire leafspot and the angular leafspot occurring in other sections of the United States, and from the black rust occurring in Sumatra, all of which are bacterial in nature. The symptoms of the wildfire disease and of the Wisconsin leafspot, however, are much the same.

The disease usually manifests itself by round, brown, or rust-colored spots, usually less than ½ inch in diameter, but frequently running together to form larger irregular lesions. Frequently the young lesions are marked by a distinct chlorotic area or halo surrounding the point of infection. Under field conditions infection usually starts on or is confined to the lower leaves. Lesions may also occur on young leaves in the seed beds.

The disease is caused by a yellow bacterial organism apparently previously undescribed. The name *Bacterium melleum*, n. sp., is suggested, and the more common morphological and cultural characteristics are given.

Artificial infection has been secured only through wounding by needle pricks. Under the conditions of the inoculation experiments in the greenhouse this has also been more or less true with the wildfire organism, which has been studied comparatively in practically all of the work done with the Wisconsin leafspot organism. Under field conditions it is not believed that wounding is necessary for infection. Temperature and humidity conditions in themselves do not apparently govern the occurrence of infection. Some data secured indicate that predisposition may be influenced by the fertilizing materials available.

It is believed that the disease ordinarily starts in the seed beds, from which it is transferred to the field. Growers are advised, therefore, not to use plants from infected seed bed areas for transplanting.

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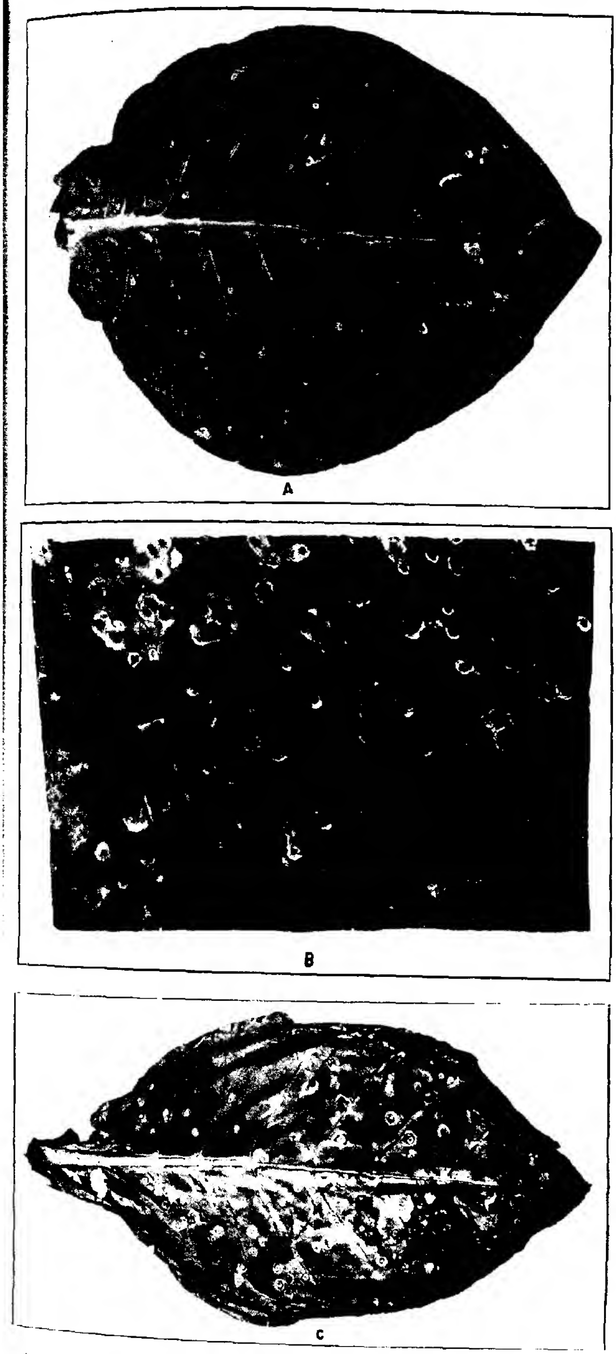
PLATE 1

A.—Leaf of Havana Seed tobacco, showing fairly typical symptoms of the Wisconsin leafspot ("rust") disease. Natural infection.

B.—More detailed view of portion of leaf similar to that illustrated in A, showing the central points of infection, the coalescence of the spots, and their relation to the veins.

C.—A leaf showing a fairly typical case of "wildfire" on Havana Seed tobacco. Compare with Wisconsin leafspot. This leaf was collected in Connecticut in July, 1919, and was the first authentic case of the wildfire disease in New England.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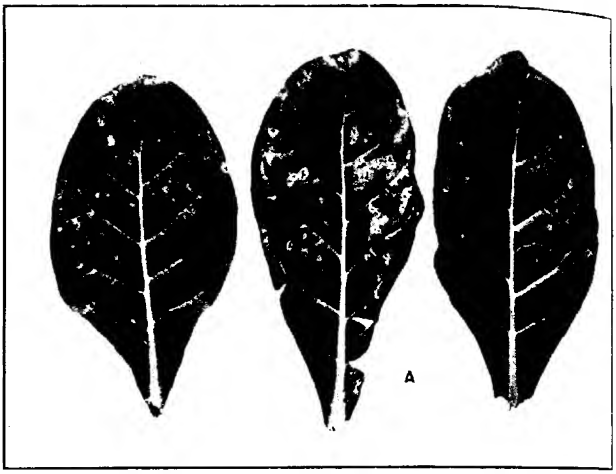
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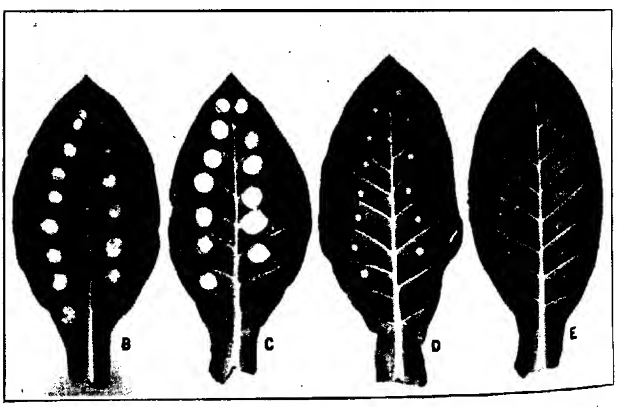

B
C
D
E

PLATE 2

A.—Leaves from tobacco seedlings in seed beds, showing typical lesions. Such leaves readily carry the infestation to the fields. Note the halo surrounding the points of infection (B, C, and D). Artificial inoculation with leafspot organisms by means of pin pricks on tobacco leaves, illustrating their similarity under certain conditions (B, C, D, and E).

B.—Leaf inoculated with Wisconsin leafspot organism.

C.—Leaf inoculated with wildfire organisms.

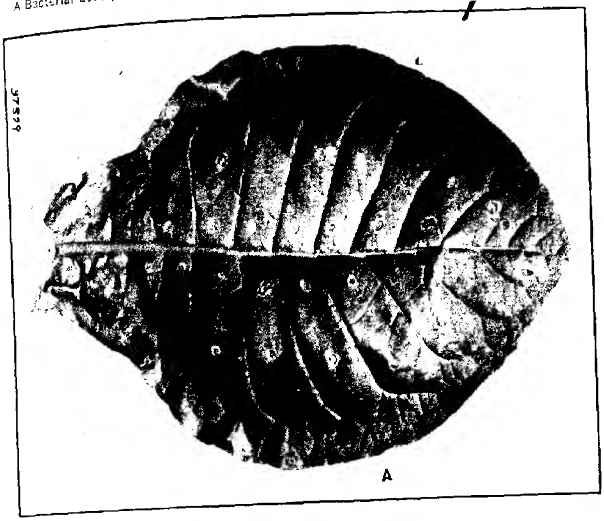
D.—Leaf inoculated with Wisconsin leafspot organism with lowered virulence.

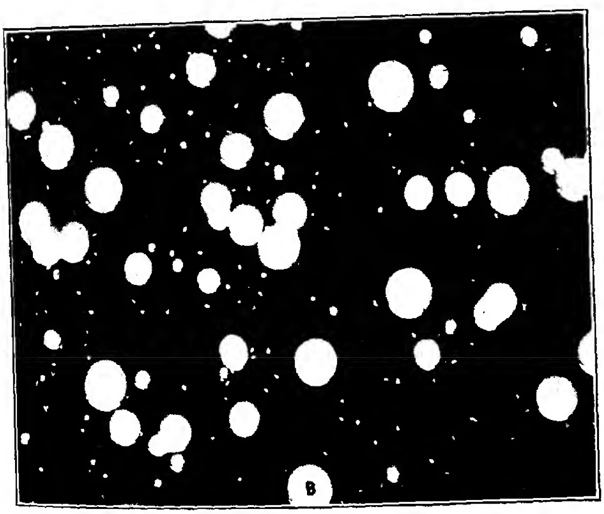
E.—Control, pricked, but not inoculated.

PLATE 3

A.—A leaf of tobacco inoculated with the Wisconsin leafspot organism by means of pin pricks. Tissue collapsed rapidly around point of inoculation and turned brown, no halo developing. Similar spotting without halo may also develop in the wildfire disease.

B.—Typical colonies of the Wisconsin leafspot organism on potato agar plate.





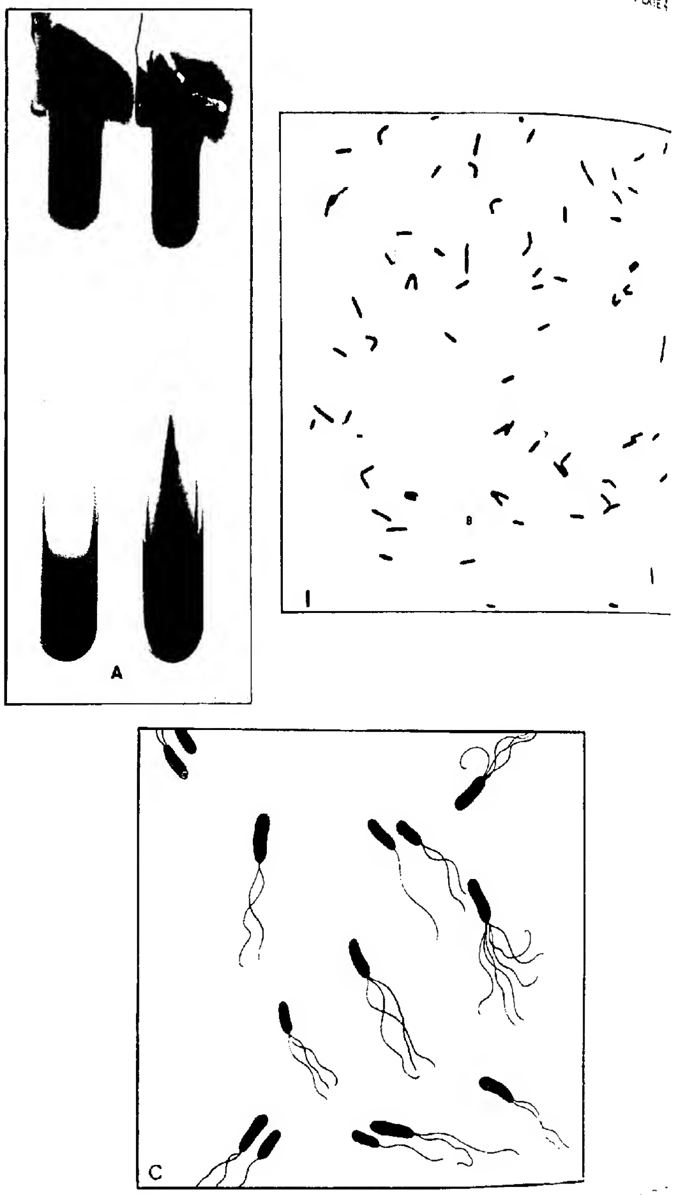

A
B
C

PLATE 4

A.—Streak of Wisconsin leafspot organism on potato-dextrose agar after four days' growth at 20° to 22° C. in comparison with control tube. Note color difference of culture medium, as a result of development of yellow pigment on potato-dextrose agar.

B.—Photomicrograph of Wisconsin leafspot organism. Carbolfuchsin stain. ×3,000.

C.—Line drawing of Wisconsin leafspot organism, showing flagella.

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